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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FORTY-FIRST ANNUAL MEETING OF THE SOCIETY, NEW YORK CITY, NEW YORK, DECEMBER 28 TO 30, 1949

Effect of tobacco mosaic virus on yield of glasshouse and field grown tomatoes. ALEXANDER, L. J. Two experiments have been conducted to determine how much the tobacco mosaic virus reduces yield of tomatoes. Plants in the unstaked control plot remained free of the virus, but 13.2 per cent of plants in the staked plot were infected when the first fruit was picked. Thereafter no further effort was made to limit spread of the virus. Yields from plants in staked plots were reduced 0.5, 7.2, 8.1, and 11.0 per cent and those from plants in unstaked plots were reduced 17.0, 16.0, 21.8, and 26.4 per cent, respectively, for the July 16, June 18, May 29, and May 10 dates of inoculation. For the same dates of inoculation the respective decrease in number of fruits per plant from staked plants was 5.4, 11.3, 10.2, and 17.9, and for plants in unstaked plots, 1.9, 0.2, 4.4, and 11.8. In a second experiment conducted in a commercial glasshouse, the yield in terms of pounds per plant was 11.80, 12.96, 13.20, and 13.40, respectively, from plants inoculated January 18, February 23, March 29, and April 12. In terms of dollars the increase would be considerably greater.

Relation of time of day and other factors in mass inoculation with Xanthomonas pruni. ANDERSON, H. W., AND DWIGHT POWELL. Three-year-old Elberta peach trees free of bacterial spot were inoculated with *Xanthomonas pruni* at different hours. Forced stream and mist sprays were contrasted. Three series of the time-of-day inoculations were made and one series on forced stream versus mist sprays. Typical results on time-of-day based on counts of infected dropped leaves per tree 18 days after inoculation were: 6 a.m., 22; 11 a.m., 517; 2 p.m., 196; 5 p.m., 283; 10 p.m., 65; check, 0 (June 2 and 3 inoculations). Subsequent counts of fallen leaves and infected leaves on trees showed the same relationship. Mist-spray inoculation compared with forced stream gave respectively 31 and 1342 dropped infected leaves per tree within a 26-day period (July 1 inoculations). Somewhat higher infections in the three series resulted from inoculations on hot days than on cool. No rain fell within 24 hr. of the inoculations, the soil was moderately dry, and sun was bright. Infection usually occurred on 3–6 adjacent leaves per shoot where the shoots had 12–16 leaves. The stage of development of the infected leaves varied in the three series. Infection did not occur on young unfolded leaves.

Wilt-inducing polysaccharides of phytopathogenic bacteria. ARK, PETER A., AND WILLIAM A. FEDER. A polysaccharide-lipide complex of *Agrobacterium tumefaciens*, *Xanthomonas phaseoli*, and *Erwinia carotovora* prepared by the trichloracetic acid extraction method and combined with water induced wilt in cuttings of a number of plant species. The polysaccharide and lipide portions are separated by acetic acid hydrolysis of the complex. The polysaccharide portion is an active wilt-inducing agent. The polysaccharide differs in yield and optical rotation from that described by Riker *et al.*

Method of inoculation and the production of spores by Guignardia bidwellii and other fungi in culture. BARNETT, H. L., MARGARET B. TIMNICK, AND VIRGIL G. LILLY. *Guignardia bidwellii* was used as a test fungus in a study of the factors which influence sporulation in culture. Inoculation of favorable agar media, either with mycelium or a few conidia at one point, resulted in vigorously growing mycelium which produced large pycnidia containing abundant conidia after 10 to 20 days. When media were flooded with a heavy suspension of conidia, little aerial mycelium was produced, but the entire surface was covered with conidia after 3 days. These conidia were borne directly on the mycelium without the formation of pycnidia. The use of a suspension of finely cut mycelium gave essentially the same result. Leaching of nutrients from cultures by continuous flow of water resulted in rapid production of conidia on germ tubes and young mycelium, while a continuous flow of nutrient solution delayed or prevented sporulation. It was concluded that the competition resulting from a multiplicity of growing points on agar media soon depletes the supply of nutrients, making conditions unfavorable for further vegetative growth yet favorable for sporulation. Under these conditions, these changes are so rapid that the formation of definite pycnidia is by-passed. *Glomerella cingulata*, *Endothia parasitica*, and certain other fungi tested responded in a similar way.

Aquatic Oomycetes of the vicinity of Orono, Maine. BARTEAUX, MIRIAM MARSH. An investigation of the aquatic Oomycetes of the vicinity of Orono, Maine has been

made. Three species isolated are thought to be parasites on fish, while six are considered to be parasites on other animal and plant substrata. Water samples were collected from the rivers, streams, stagnant pools, springs, drainage ditches, and a sphagnum bog. Soil samples were obtained from miscellaneous sources. Hemp seeds and dead bees were used as solid nutrient. Hyphae were transferred to sterilized media. Hyphae were in turn transferred to agar plates containing cornmeal-dextrose or pea agar. For storage, mycelia were transferred from agar plates to tubes containing the same nutrient agar and submerged in extra heavy mineral oil. A few grains of dextrose added to the culture medium prolonged the asexual stage. Twenty-six species have been identified: *Allomyces arbuscula, Pythiopsis cymosa, Saprolegnia anispora, S. ferax, S. monoica* var. *glomerata, S. parasitica, S. lapponica, Achlya americana, A. Klebsiana, A. racemosa, A. flagellata, A. hypogyna, Protoachlya paradoxa, Aphanomyces laevis, Dictyuchus monosporus, Geolegnia septisporangia, Gonapodya polymorpha, Sapromyces Reinschii, Pythium arrhenomanes, P. catenulatum, P. deBaryanum, P. hypogynum, P. proliferum, P. mamillatum, P. pulchrum, P. torulosum.*

Retention of copper on potato foliage as an index for protection against disease. BASKIN, A. DAVID. In copper retention studies on potato foliage (1947–49), Bordeaux (8–8–100) spray consistently showed higher deposits of copper than all other copper fungicides tested. Copper fungicides applied as sprays always resulted in higher initial residues than when the same materials were applied as dusts. The rate of weathering of spray deposits was slower than that of dusts. Some proprietary stickers improved the retention of tribasic copper sulfate spray almost to the equal of Bordeaux. However, as observed in 1948, dusts applied at regular intervals, particularly after a period of rain, effectively reduced defoliation due to late blight. Late blight occurred only in 1948, and that year there was some indication that control of late blight paralleled copper adhesion. Higher copper adherence will not result in better late blight control unless fungicide applications are continued as long as weather conditions favor late blight.

Cultural and pathogenicity studies of anthracnose of cereals and other grasses. BELL, FRANK H. Single-spore isolates of *Colletotrichum graminicolum* were obtained from 72 collections of diseased wheat, rye, oats, barley, and several other grasses, principally from Ohio. Culture mycelia of the cereal isolates were nearly all of a dark type. Most of those from noncereal grasses were either a light or an intermediate type. Light-colored overgrowths were common on the older culture mycelia. Many single-spore variants differing in cultural characteristics were obtained from these overgrowths. Many of these were more stable, and a few produced more spores, than the original isolates. Nearly all isolates from rye and wheat caused seedling blight of rye and wheat, but not of Clinton oats and Kentucky No. 1 barley in greenhouse pathogenicity tests. Most of the isolates from noncereal grasses did not cause appreciable seedling blight. Of the wheats, Butler and Vigo were the most resistant to seedling blight, and Blackhawk, Cornell 595, and Trumbull were generally the most susceptible. Some of the cultural variants were more pathogenic than their "parent" isolates. A few were nonpathogenic.

Filtration and sedimentation of wound-tumor virus. BLACK, L. M., KARL MARAMOROSCH, AND M. K. BRAKKE. The wound-tumor virus may be detected in solutions freed of host cells by injecting small amounts into vector leafhoppers and subsequently testing the ability of the insects to infect plants. Using this technique it has been ascertained that the virus will pass through Berkefeld N and W filters retaining *Serratia marcescens.* By testing for infectivity at different depths in solutions spun horizontally in capillary glass tubes, it has been estimated that the virus has a sedimentation constant at 20° C. of about 600 Svedberg units. Although the virus is smaller than potato-yellow-dwarf virus, it is considerably larger than other plant viruses of known size. Juice from infected crimson clover has proved infectious at a dilution of 10^{-3}; from infective leafhoppers at a dilution of 10^{-5}. Residual active virus is present in neutral solution after treatment at 50° C. for 10 min., after storage at 0° C. for 48 hr. and after freezing at −25° C. for 48 hr. Some activity remains after storage for 1 hr. at 0° C. at pH 4.0 or 10.7. None of these values can at present be regarded as the extremes which the virus will survive.

A partial-vacuum technique for inoculating seedlings with bacteria and fungi. BOOSALIS, MICHAEL G. More than 1,000 seedlings of wheat, oats, barley, rye, and *Bromus inermis* have been inoculated quickly and effectively with *Xanthomonas translucens* f. sp. *undulosum* by the following method. Strips of newspaper or blotting paper, 6×2 in., were folded lengthwise and 30 seeds were placed in the fold and covered with vermiculite. Blocks of 7 to 10 seeded strips were held together by rubber bands. Germinating seeds were kept at room temperature (20°–25° C.) and watered daily with tap water. When

the seedlings were 3 to 5 in. high, 7 blocks were submerged in a water suspension of bacteria in a pressure cooker. Evacuation to approximately 0.008 mm. mercury for 1 to 5 min. was attained by means of a Cenco Hyvac pump. The inoculated seedlings were then grown in soil or in vermiculite supplemented with nutrient solution. Removal of paper was unnecessary because roots easily penetrate it. By use of this method 100 per cent infection usually was evident within about 5 days. The method, or a modification of it, may be used with certain smut fungi.

Studies on the action of copper seed treatments for water-planted rice. BOUCHEREAU, P., AND J. G. ATKINS, JR. Laboratory studies were made in Louisiana as to the action of copper fungicides, particularly yellow cuprocide, previously shown to give increased stands of presprouted, water-planted rice, as seed treatment under conditions of continuous flooding. Soil-infesting fungi were found to be more important than seed-borne fungi. Although germination of seed was not increased by treatment with yellow cuprocide, seedling survival was increased in tests using *Helminthosporium oryzae* or naturally infested soil from rice fields. Treatment increased root and shoot growth. Rice treated with yellow cuprocide at 1, 2, and 4 oz. per bu. was stored and tested at monthly intervals. Roots and shoots of seedlings grown from treated seed had a higher metallic copper content than those from untreated seed. Prolonged storage resulted in greater differences. Roots and shoots of seedlings grown from treated seed that had been stored for 7 months contained, respectively, 8 and 9 times as much copper as those from untreated seed.

Correlation of potato yellow-dwarf virus bodies with infectivity during centrifugation. BRAKKE, M. K., L. M. BLACK, AND R. W. G. WYCKOFF. The first electron-micrographs of a leafhopper-transmitted plant virus, those of potato yellow-dwarf, were published by Black, Mosely, and Wyckoff. The bodies illustrated are several times larger than those known for any other plant virus. To obtain more evidence on the size, the sedimentation rate has been determined by horizontal centrifugation in 2-mm. glass capillary tubes. A boundary was visible in tubes containing a partially purified preparation of the virus from *Nicotiana rustica* but not in tubes containing a corresponding extract from healthy plants. Upon extrapolation to zero concentration of solids in the extracts, a value of 1130 ± 100 Svedberg units was obtained for the sedimentation constant of the boundary, corrected to water at 20° C. This corresponds to a particle approximately the size of the bodies seen in the electron micrographs, *i.e.*, about 110 $m\mu$ diameter. Samples removed from below the boundary were infective and contained the bodies seen in the electron micrographs, while those removed from above the boundary were not infective nor did they contain the bodies. Since this procedure can be used with impure preparations and requires only a qualitative assay, use of it in combination with the insect injecton technique should provide information about the size of other leafhopper-transmitted plant viruses.

Thermal inactivation studies on the tumor-inducing principle in crown gall. BRAUN, ARMIN C. The thermal inactivation of the tumor-inducing principle in crown gall was investigated according to methods recently developed. The studies were carried out at intervals of 0.1° C. between 25° and 30° C. Two criteria, (1) size and weight of tumors produced in 21 days from cells transformed to tumor cells in 24 hr. at the desired temperature, and (2) delay of inception period, were used in establishing inactivation rates. *Kalanchoe* was the test plant. The results demonstrated that 25° to 27.4° C. approached the optimum for cellular transformation. Between 27.5° and 28.8° C. there was a progressive decrease in the size and weight of the resulting tumors and a delay in the inception period. Tumors were not initiated above 29° C. Measurable inactivation was thus limited to less than 2° C. Reaction rates could nevertheless be established and the energetics of inactivation studied. When the activation energy was computed according to the Arrhenius equation $Ea = \frac{RT_1T_2}{T_2-T_1} \ln \frac{K_2}{K_1}$ from data obtained at 27.3° and 28.6° C., very high values of more than 80,000 calories per mole were found. Since reactions of this order of magnitude are characteristic of protein denaturation, the results suggest that either the tumor-inducing principle itself or something intimately associated with its inactivation is a factor of complex structure.

Seed transmission of the elm mosaic virus. BRETZ, T. W. Hand-picked seed from a mosaic-infected American elm was planted in the greenhouse and the seedlings obtained were examined periodically for mosaic symptoms. Prior to planting, the seed was divided into two lots; in one the seed was separated from the fruit covering; in the second the fruit covering was left intact. Over a 3-month period, approximately 1 per cent and 3¼ per

cent, respectively, of the seedlings developed characteristic mosaic symptoms. Because approximately 20 per cent of the fruit was observed to be malformed, another planting of malformed versus normal fruits was made to determine whether a correlation existed between the appearance of the fruit and mosaic symptom expression. There appeared to be no such correlation. The percentage of seedlings showing mosaic symptoms was approximately the same in each lot. When leaf tissue from seedlings having mosaic symptoms was grafted into healthy elm seedlings, about 25 per cent of the grafted seedlings developed typical mosaic symptoms within 5 months. Seedlings of the same lot in a control series remained healthy.

Experimental propagation of disease-resistant elm selections by vegetative cuttings. BRETZ, T. W., AND R. U. SWINGLE. Research on the development of elms resistant to the Dutch elm disease and to phloem necrosis has included methods for the propagation of disease-resistant trees by vegetative cuttings. Requirements for the production of rooted elm cuttings varied between species and between individuals of the same species. The Christine Buisman elm, selected from *Ulmus carpinifolia* and resistant to Dutch elm disease and phloem necrosis, was propagated best on its own roots from root cuttings collected during the dormant season. Selections from *U. americana* were propagated most satisfactorily from softwood stem cuttings or leaf-bud cuttings with a "heel" of stem tissue collected in early summer and subjected to a constant water mist. The highest percentage of root strike and the most vigorous rooting was obtained with leaf-bud cuttings treated with rooting compounds. Survival of rooted leaf-bud transplants was greater than the survival of rooted softwood stem cuttings. Dormant stem cuttings gave generally unsatisfactory results. *U. thomasi, U. pumila,* and *U. fulva* were propagated successfully by means of leaf-bud cuttings, and preliminary trials using this technique with hybrid Castanea have given promising results.

Orchard tests with a large air-blast sprayer modified to apply partial concentrates. BURRELL, A. B. Large, non-replicated plots in a 17-year-old McIntosh apple orchard were sprayed throughout the season with usual fungicide-insecticide combinations at 2, 4, and 8 times standard concentrations. Gallonage was reduced proportionately, so the same amount of chemical was directed toward each tree. The machine was a 1947 F.M.C. Speedsprayer operating at 60 p.s.i. water pressure, 45,000 cu. ft. of air per minute at 95 m. p. h. when delivering both sides. Special liquid nozzles giving finer break-up and shields to modify volume, velocity, and direction of air-blast were provided. Agitation in Speedsprayer was barely adequate to mix the heaviest combination consisting of 40 lb. sulphur, 24 lb. lead arsenate, and 24 lb. lime per 100 gal.; however, it readily applied this combination when premixed. Nozzles such as the Whirljet, Flatjet, and Speedmist, being free of internal obstructions, minimize clogging. Control of apple scab and certain insects was satisfactory and similar with all concentrations. Interference with fruit color-development decreased as spray concentration increased because the spray did not flow into large drops. Coverage of roughbarked branches offers special problems for concentrates. To obtain greater speed and economy certain large air-blast sprayers designed for standard formulas may be adapted to application of somewhat concentrated mixtures.

Assaying chemotherapeutants in the greenhouse. CHAPMAN, R. A., A. E. DIMOND, AND E. M. STODDARD. In chemotherapy experiments conducted in the greenhouse with Fusarium wilt of tomatoes, infection has been consistently prevented. Moreover, differences between compounds and between different concentrations of the same compound have been determined with a high degree of significance. Plants in sand are grown at temperatures and nutrient levels optimal for disease development. The test compound is applied to the sand and must be absorbed by the plant to be effective. Then plants are inoculated by uprooting them, washing, wounding, and dipping the roots in a suspension of the pathogen. After inoculation, they are replanted in clean sand in pots. Such methods have shown that effective compounds will prevent but not cure Fusarium wilt of tomatoes. Disease readings are made by indexing 3 components of the syndrome: (1) foliage wilting and yellowing, and discoloration of vascular bundles of (2) petiole and (3) stem, the last index being the most consistent. Differences between foliage and petiole indices are a measure of toxin activity in the production of foliage symptoms. We have not been able to recover the fungus from symptomless plants or plant parts.

Predicting protective value of fungicides from slope of wash-off curve. CHAPMAN, R. A., JAMES G. HORSFALL, AND H. L. KEIL. Field performance of candidate fungicides can be predicted in the laboratory from biologically measured slopes of wash-off curves (S_w). This is true when other weathering factors are not limiting. Wash-off

data were obtained from mortality counts of spores of *Sclerotinia fructicola* and *Stemphyllium sarcinaeformae* placed on differentially washed slides which had the same initial deposit of toxicant. Slopes were measured from standard log-probit plots. Measured in this way, S_w is primarily the resultant of slope of dosage-response curve and physical resistance to washing. Flat S_w predicts good field performance. Field performance against apple scab and black spot of roses was predicted on the basis of S_w of a series of analogues of bis (2-hydroxy, 5 chlorophenyl) sulfide (Cr. 305) in which the slope of the dosage-response curve varied widely. When apple-scab indices were plotted against S_w for *S. fructicola* and for *S. sarcinaeformae*, highly significant correlation coefficients of 0.856 and 0.670, respectively, were obtained. The better correlation in the first comparison is expected since Horsfall and Rich (1950) have shown that Cr 305 is toxic to *S. fructicola* and *Venturia inaequalis* and not to *S. sarcinaeformae*.

Leaf spot of Crotalaria caused by Septoria. CLAYTON, C. N., AND J. LEWIS ALLISON. Crotalaria is an important root-knot nematode-resistant legume grown as a cover crop on many peach orchard soils in the Sand Hill area of North Carolina. A leaf-spot disease was first noted attacking cotyledonary and basal leaves of *C. spectabilis* seedlings in June, 1948. Infected leaves became yellow and dropped. As the season progressed, the epiphytotic increased in intensity and by late September average plant defoliation was estimated as 85 per cent. The leaf spot was found on leaves of *C. mucronata* var. Giant striata but caused less defoliation of plants of this species. The disease was prevalent in the area again in 1949. The lesion is necrotic and circular (1 to 3 mm.) with a reddish brown margin and a tan to almost white center in which one to several pycnidia are visible. A fungus of the genus *Septoria* was consistently associated with and isolated from lesions. Typical symptoms were produced on leaves of seedling plants of *C. spectabilis* when inoculated under controlled conditions, and the fungus could be readily reisolated. This is the first record of a leaf spot on *Crotalaria* caused by a species of *Septoria* and in all probability the fungus constitutes a new species.

Study of disease resistance from interspecific crosses. CLAYTON, E. E. High level resistance to major tobacco diseases, not found in the cultivated species (*Nicotiana tabacum*), occurs in distantly related wild species. Utilization of this resistance through interspecific crosses is frequently hampered by sterility. The direction of crossing has proven a major factor in the development of sterility. *N. debneyi* and *N. tabacum* were crossed. The latter was the male parent in the original cross and in subsequent backcrosses. Sterility increased until in backcross 3 all progeny were completely male sterile. This sterility was prevented by using *N. tabacum* as the female parent in backcross 1 or 2. Identical results were obtained with the *N. megalosiphon-N. tabacum* combination. Progress after interspecies crossing requires the establishment of resistant lines both stable and fertile. Two types of such lines have been secured: (1) Substitution lines in which tobacco chromosomes are replaced by chromosomes from the wild species. These lines segregate irregularly in the F_2. (2) Translocation lines in which the resistance genes from the wild species are included in tobacco chromosomes. These segregate regularly in the F_2. Results indicate that cultivated tobacco can be greatly improved by transfer of immunity to blue mold and black root rot from *N. debneyi* and of similar high level resistance to wildfire, blackfire, and black shank from *N. longiflora*.

Pathological anatomy of internal bark necrosis of apple. CLULO, GENEVIEVE. Specimens were collected from trees grown in the orchard and greenhouse. The necrotic lesions are initiated early in the growing season but they usually do not manifest themselves externally until near the end of the growing season or even until the end of a second growing season. Necrotic lesions in older bark may be initiated in any group of parenchyma cells from cambium to outermost cells of the cortex. In severely diseased trees where stem tips of the current season's growth are involved, lesions are found in all parenchyma tissue, even that of the xylem parenchyma, from pith to cortex. Lesions are usually numerous in leaf and branch gaps and on bud scales. The cells involved in necrotic lesions undergo many complex changes both in behavior of nuclei and cytoplasm. Cell division is increased and finally many cells divide so rapidly that cell walls are not laid down and often a single cell may contain two or three degenerated nuclei. A well-developed layer of cork cells forms around each lesion or group of closely associated lesions.

Direct observation of formation of sexual bodies by combination of hyphae of two Phytophthora isolates. COHEN, MORTIMER. Formation of sexual bodies has been studied in crosses between an isolate of *Phytophthora palmivora* and one of *P. cinnamomi*. Small pieces of mycelium of these two species were placed a short distance apart on a

thin layer of tomato-juice agar on a cover slip inverted over a Van Tieghem cell. Sexual bodies were formed where hyphae of the two came into contact with one another. In this cross the *P. palmivora* isolate formed oogonia, the *P. cinnamomi*, amphigynous antheridia. The hyphae bearing the gametangia could be traced to their parent mycelia. Definite morphological differences between the two species aided identification of their hyphae. This constitutes additional evidence of heterothallism in this genus.

Two virus diseases of peanuts. COOPER, W. E. Two virus diseases of peanuts designated as "mild" and "severe" mosaic, have been found in North Carolina. The mild mosaic consists of faint chlorotic mottle or spot patterns. Stunting and leaf distortion are slight. The disease appears on a few scattered plants early in June, and by August nearly all plants in some fields show symptoms. It occurs throughout northeastern North Carolina on Virginia Bunch and Virginia Runner peanuts. Its effect on yield is not known. The severe mosaic is characterized by an intensive chlorotic, sometimes necrotic, pattern with severe stunting and distortion of affected leaflets, and marked stunting if plants are infected when young. It usually occurs on isolated plants, although indications of field spread have been observed. Both diseases have been transmitted to peanut by the carborundum-rubbing method and by cleft grafts. A modified cleft graft was used in which scotch tape was closely folded around the union. This technique is fast, effective, and well adapted to herbaceous plants like peanuts. Preliminary studies indicate that these diseases are transmitted by aphids. Whether these two diseases are similar to or identical with those reported from other countries is not known.

Cultural characters of uranium-nitrate-induced mutants of Ustilago zeae. DALY, J. M., AND S. I. LU. A study was made of 13 characters of 198 mutants from monosporidial, unisexual line 17D4 of *Ustilago zeae*. Ten of these appeared in colonies of 17D4 on potato-dextrose agar plus uranium nitrate; 173 were mutants from the 10 original mutants, also growing on uranium-nitrate medium; and the remaining 15 appeared in colonies of some of the 173 mutants growing on uranium-free medium. Plus or minus deviations from 17D4 in color, topography, radial growth, rate of growth, elevation, thickness, luster, toughness, zonation in color and topography, characters of margin of colonies, and in tendency to mutate were studied under uniform conditions on a synthetic medium. Based on algebraic summation of plus and minus deviations in each of the 13 characters of all mutants, about 20 per cent of the total population exceeded 17D4, 20 per cent were inferior, and the remaining 60 per cent were approximately equal to 17D4. Individual mutants often displayed both gain and loss, but there was neither pronounced gain nor loss in the totality of characters of the entire population.

Helminthosporium seedling blights of corn. DAVIDSON, R. S. Three species of *Helminthosporium* which cause leaf blight of corn also produce corn seedling blight. *H. turcicum*, *H. maydis*, *H. carbonum* race I, and *H. carbonum* race II, are pathogenic on corn seedlings, their general virulence increasing in the order given. Different corn hybrids vary in their susceptibility to all species studied; but the pathogenicity of the species and races is in the same order for each hybrid studied. All species listed injure the mesocotyl and stunt the seedlings. *H. carbonum* race I produces a slight amount of seedling blight, whereas *H. carbonum* race II produces severe seedling blight. Different isolates of *H. carbonum* race II vary in pathogenicity. The optimum soil temperature for pathogenicity of all species studied was 20° to 28° C., and yet *H. carbonum* race II was pathogenic at soil temperatures as low as 10° C.

Factors affecting the susceptibility of certain winter wheat varieties to scab infection. DAVIDSON, R. S., AND DEN-SHEN TU. Flowering habits of four soft red winter wheat varieties—Butler, Thorne, Trumbull, and TN–1016–4—were observed in the field to determine a possible relation between flowering habit and susceptibility to wheat scab, *Gibberella zeae*. Extensive field observations over several years in Ohio have shown Trumbull to be the wheat variety most resistant to head blight infection. Individual florets of the four varieties were examined to determine the length of time they remained open. Forty-eight per cent of the florets of Trumbull remained open for more than 18 min., whereas 85, 74, and 59 per cent of the florets of TN–1016–4, Thorne, and Butler, respectively, remained open for the same period. Florets which open before 8 a.m. and those which open after 4 p.m. are more numerous in Thorne, TN–1016–4, and Butler than in Trumbull. In Trumbull, 28 per cent of the florets retained anthers, whereas 44, 50, and 68 per cent of the florets in Thorne, TN–1016–4, and Butler, respectively, retained anthers.

Control of foliar nematode disease of chrysanthemums with parathion sprays. DIMOCK, A. W., AND C. H. FORD. During the 1949 season field tests were conducted at Ithaca and Farmingdale, N. Y., to assess the value of various insecticides as foliage sprays for the control of chrysanthemum leaf nematode (*Aphelenchoides ritzema-bosi*). Twenty-plant plots, at Farmingdale, and 10-plant plots, at Ithaca, were set out in four randomized blocks. Spray applications were made at intervals of approximately 7 days. Infected-leaf inoculum was scattered uniformly through all plots two or three times during the season and overhead irrigation was employed frequently. Spectacular control was obtained with parathion in both locations. At Farmingdale, the total infected-leaf count for all plots was reduced from 1296 in the checks to 1 and 0 for parathion wettable powder at 0.25 and 0.50 lb. active per 100 gal., respectively. At Ithaca, near-perfect control was also obtained with parathion wettable powder at 0.25 lb. active per 100 gal. and good, but somewhat less effective, control with parathion emulsion at 0.17 lb. active per 100 gal. None of the other materials tested, including chlordane, benzene hexachloride, tetraethylpyrophosphate, DDT, and nicotine sulphate, approached this degree of control.

Effects of continuous gamma radiation on tomato plants and Fusarium wilt. DIMOND, A. E. Under contract with the Atomic Energy Commission, the Connecticut Agricultural Experiment Station is investigating therapeutic value of nuclear radiations in diseased plants. At Brookhaven National Laboratory, Bonny Best tomatoes and cultures of *Fusarium lycopersici* were exposed continuously to a 20 rhm source of Co^{60} gamma radiation. Spores from cultures of *Fusarium lycopersici* exposed to 32,000 r in 2 months showed a mortality less than 15 per cent. When dosage was less than 50,000 r, tomato plants receiving 150 r/hr grew only slightly while exposed, but normally after irradiation ceased. Above 50,000 r, plants remained dormant, showing severe radiation injury. Slight terminal growth and lack of flowering at high dosages results in tiny plants. Reduced fruit set and unaffected terminal growth at intermediate dosages results in very large vines. Normal fruit set and vegetative growth at low dosages results in plants of intermediate size. No parthenocarpy was observed. Irradiated plants, subsequently inoculated, showed typical Fusarium wilt. Inoculated tomatoes, subsequently irradiated, died of Fusarium wilt at all intensities employed. W. R. Singleton, D. Balber, F. German, and J. Connelly, all of Brookhaven National Laboratory, were of great help in this study.

Relation between Argentina's lepra explosiva and Florida's scaly bark of citrus. DUCHARME, E. P., AND L. CARL KNORR. Uncertainty has existed concerning the relationship between South American "lepra explosiva" and "Florida scaly bark" of citrus. Serious losses in Argentina are being caused by lepra, and centers of scaly bark currently exist in Florida. Field comparisons in Argentina and Florida reveal such fundamental similarities with respect to symptoms on bark, fruit, and leaves as to lead to the conclusion that lepra and Florida scaly bark are synonymous. Minor variations exist between the diseases; these, however, are more apparent than significant, being considered effects of environmental differences. Opportunity is taken to urge adoption of the name "leprosis" in preference to the earlier Floridian terms "scaly bark" and "nailhead rust," and the South American terms "variola" and "lepra explosiva."

Control of anthracnose canker of Camellia. DUNLAP, A. A. Records were taken in 1949 of surgical treatment applied in 1940 and 1941 to *Camellia japonica* in ornamental plantings affected with the canker disease caused by the fungus *Glomerella cingulata*. The treatment consisted in removal of dead or dying twigs and discolored wood around cankers on the limbs and trunks. This surgical work was followed by application of an asphaltum-fungicide paint. Additional spray applications of an insoluble copper compound plus a glue spreader-sticker were occasionally made following the surgery. In most cases treatments were successful in checking growth of cankers, and treated plants have remained apparently healthy for 7 or 8 years. Callus growth has been generally slow and many larger wounds still remain open with healthy margins and sound heart wood.

"Blackpatch" as a limiting factor in red-clover seed production in West Virginia. ELLIOTT, EDWARD S., AND J. G. LEACH. The "blackpatch" fungus, heretofore known to be parasitic only on leaves of certain legumes and to cause only minor damage to red clover, has been found to be strongly pathogenic to stems and flowers of red clover and to be a limiting factor in seed production in West Virginia. The disease was preva-

lent on both first- and second-year clover but was most destructive on the second-year seed crop in which the stems and flower heads were affected. The greatest damage resulted from the girdling of the stem beneath the flower head before the seeds were mature, or from direct infection of the flowers before the seeds were fully formed. Injury was most severe from midsummer to fall and was favored by rainy weather and high humidity. Pathogenicity of the fungus to stems and flowers has been confirmed by artificial inoculations under controlled conditions. No sporulation of the fungus has been observed, but it has been isolated from one-year-old clover seeds, indicating that it may survive the winter in or on seeds from infected plants.

Etiological and control studies on lettuce damping-off in North Carolina. ELLIS, D. E., AND R. S. COX. Damping-off in the plant bed is an important limiting factor in the production of head lettuce in southeastern North Carolina. Surveys and isolations show that *Rhizoctonia solani* and *Pythium* spp. are the pathogens most consistently associated with the disease. Species of *Sclerotinia* and *Botrytis* are occasionally encountered. In greenhouse inoculation tests, *R. solani* consistently caused both pre- and post-emergence damping-off, while *Pythium* spp. caused pre-emergence damping-off and root rot but rarely produced typical post-emergence damping-off. Of 59 isolates of *Pythium* spp. tested, 17 caused pre-emergence damping-off, 1 caused post-emergence damping-off, 27 caused root rot which resulted in stunting, 11 caused slight root rot but no stunting, and 21 were nonpathogenic. Of 24 of the more highly pathogenic isolates, 17 were *P. debaryanum*, 3 were *P. spinosum*, and 4 were tentatively determined as *P. irregulare*. Seed treatment with Spergon (tetrachloro-parabenzoquinone) or Cuprocide (red cuprous oxide) followed by drenches of the carbamate Fermate (1–100) or of the thiuram-disulfide Thiosan (1–100) drenches applied at 10-day intervals at ½ gal. per sq. yd. gave effective and practical control. Drenches with several organic mercury and copper compounds resulted in partial control but were phytotoxic.

The effect of zinc ethylene bisdithiocarbamate on the growth responses of the tomato plant. EMGE, ROBERT G., AND M. B. LINN. Tomato seedlings were grown in a modified Hoagland solution in which only the zinc content was varied. A zinc ethylene bisdithiocarbamate (zineb) suspension and a zinc sulfate solution were sprayed on plants growing in zinc-deficient and in complete nutrient solutions. Growth responses were determined on the bases of dry weight, height, and, in some instances, zinc content of the plants. The dry weight and zinc content of plants growing in a zinc-deficient solution and sprayed with zineb were comparable to those of unsprayed plants in a complete nutrient solution. The dry weight of plants growing in a complete nutrient solution and sprayed with zineb was greater than that of unsprayed plants also in a complete nutrient solution. When plants with severe zinc-deficiency symptoms were sprayed with zineb the plants resumed growth free from zinc-deficiency symptoms. There were no significant differences in any experiments between plants sprayed with zineb and those sprayed with zinc sulphate. It is concluded that growth responses were in all probability due to the zinc in the zineb.

A fraction of Agrobacterium tumefaciens antagonizing the auxin effect on growth and respiration. FEDER, WILLIAM A. A nonproteinaceous polysaccharide-lipide complex has been prepared from dried cells of *Agrobacterium tumefaciens* by the trichloracetic acid method of Boivin *et al.* This fraction is inactive in the pea and Avena growth tests, but when added to indole-3-acetic acid (IAA), inhibits its growth-promoting properties. When bean seeds are dusted with a mixture of this fraction and IAA and allowed to germinate, the inhibitory effect of the IAA upon root and shoot growth is overcome. The fraction appears to stimulate respiration of the pea stem, but when added to IAA the stimulatory effect of the IAA upon pea stem respiration is markedly reduced.

Chemicals for Dutch elm disease therapy. FELDMAN, A. W., NESTOR E. CAROSELLI, AND F. L. HOWARD. Chemicals selected after evaluation in laboratory and greenhouse were tested in 1948 and 1949 on more than 1200 trees of *Ulmus americana* growing under natural conditions. Results were: (1) Hydraulic soil impregnation with lime suppressed wilt symptoms for at least one month when soil pH was maintained at 7.0 or slightly above. (2) Low magnesium lime gave better disease control than high magnesium lime. (3) Hydraulic soil impregnation with urea, salicylate, and azo dye, alone or combined, did not effectively suppress symptoms. These three chemicals, combined with low magnesium lime, gave approximately 70 and 50 per cent control on woodland and estate trees, respectively. Equal control was obtained when this combination was applied in the fall, spring, or summer. (4) Nutrient sprays containing urea, KH_2PO_4, and sucrose temporarily re-

tarded wilt symptoms when applied at the onset of wilting. Adding $NaNO_2$ or salicylate to the nutrient spray resulted in 40 per cent control. (5) Better control resulted when treatments were applied before rather than after inoculation. (6) Results from greenhouse elm seedlings were comparable with those in woodland experiments. (7) Trunk injections of basic chemicals ($NaHCO_3$, $KHCO_3$, $Ca(OH)_2$) gave good control; citrates of Mg, K, and Na, no control. (8) Trees succumbed more rapidly to trunk than to twig inoculation.

Control of tomato buckeye rot by copper fungicides. FELIX, E. L. Fungicidal field control of tomato buckeye rot caused by *Phytophthora parasitica* var. *terrestris* appears possible, based on two years' results at Knoxville, Tenn. In 1949, tomato plants and soil were sprayed once with 82 and 78 lb. per acre, respectively, of Cu as C-O-C-S (copper oxychloride sulfate) and tribasic copper sulphate (25 lb. to 100 gal. water plus 1 pt. of resyn adhesive no. 3605, applied at a rate of about 588 gal. an acre with 300 lb. pressure). Time was just before the fruit touched the ground. Buckeye rot in total yields was: untreated, 42.3 per cent; C-O-C-S, 6.6 per cent; and tribasic copper sulphate, 4.4 per cent (84 and 92 per cent reduction). Marketable yields in average tons per acre were: untreated, 1.66; C-O-C-S, 3.89; and tribasic copper sulphate, 3.64. These results on buckeye rot and marketable yields are very highly significant (0.1 per cent level). In 1948, dusting four times with a total of 93 lb. per acre Cu as C-O-C-S (15 per cent) resulted in much less but significant decrease of buckeye rot: untreated, 19.6 per cent, and C-O-C-S, 13.4 per cent of the total yield (32 per cent reduction). Additional evidence suggests that adherence, high pressure, and concentrated application may be important factors in the control of buckeye rot.

Prunus tomentosa as an index plant for necrotic ring spot of sour cherry. FINK, HARRY C. *Prunus tomentosa* shows promise as an index plant for cherry ring-spot virus, both in the greenhouse and in the field. In the greenhouse, virus-infected buds of sour cherry were inserted in one-year-old *P. tomentosa* at bud break. The unfolding leaves showed definite ring-spot symptoms, wilted, and died. Subsequent leaves on the same cane also expressed symptoms and died; death of the cane followed. Leaves on unbudded canes of the same plants were without symptoms. In the field, buds from trees to be indexed were inserted in *P. tomentosa* one week prior to cutting back the canes one node above the bud. Distinct ring-spot symptoms appeared on the new growth. Results of indexing on *P. tomentosa* and on peach (*P. persica*) seedlings (Hildebrand, Phytopath. 32: 712–719, 1942) were compared as follows: Of 199 *P. mahaleb* trees, 10 indexed virus-infected with *P. tomentosa*, only 7 with peach. All of 111 Early Richmond cherry trees indexed infected on *P. tomentosa*, only 41 on peach. Of 8 *P. serotina*, 4 indexed infected on both peach and *P. tomentosa*. Twenty *P. Besseyi* and 20 Waneta plums indexed virus-free on both *P. tomentosa* and peach. In no case did peach detect virus when *P. tomentosa* did not. *P. tomentosa*, a hardy, seed-grown plant, can be used for indexing throughout the year.

Spontaneous occurrence of Tilletia caries (T. tritici) on seven new grass hosts. FISCHER, GEORGE W. During 1945–1949, what appeared to be *Tilletia caries (T. tritici)* was collected in some of the western States on grasses not hitherto reported as spontaneous hosts for this smut species: *Agropyron subsecundum*, Idaho; *A. trachycaulum*, Idaho; *Bromus ciliatus*, Wyoming; *B. marginatus*, Idaho, Montana; *B. rigidus*, Washington; *B. tectorum*, Colorado, Idaho, Montana, Utah, Washington; *Poa reflexa*, Wyoming. Considerable variation in spore morphology was noted among the collections, both among the new host species and also among different collections on the same species. However, these morphological variations are similar to those existing among the 15 physiologic races of *T. caries* on wheat. *B. tectorum* is by far the most widely distributed of the bunted grasses, having been encountered in several localities of the States listed. Superficially the bunt resembles head smut, *Ustilago bullata*, which is almost omnipresent on *B. tectorum* in the West, and probably the bunt has been overlooked for many years for this reason. Spore germination of the new collections has not been studied, nor have cross-inoculations with wheat yet been attempted. *A. subsecundum* and *A. trachycaulum* were earlier demonstrated as hosts for *T. caries*, but only by inoculation with spores from naturally infected *A. cristatum* and from wheat.

Soft rot of witloof chicory caused by Pseudomonas sp. FRIEDMAN, B. A. A bacterial soft rot of witloof chicory or Belgian endive (*Cichorium intybus* L.) has been observed on the New York market for more than ten years. This salad vegetable is imported annually in quantity from Belgium. The disease starts on the outer leaves as a small, soft, light-brown lesion. Under warm and humid conditions it progresses rap-

idly, forming an extensive, mushy, dark-brown decay which finally breaks down into a foul-smelling mass. In all stages of the disease numerous bacteria are found in the tissues. A number of isolates were obtained from decayed tissue, and three purified cultures of these proved upon inoculation to be pathogenic for witloof chicory. The three isolates had the same morphological and physiological characteristics. The bacteria are Gram-negative rods, form whitish colonies, have polar flagella, and produce a green pigment in asparagine broth. A member of the genus *Pseudomonas*, this pathogen appears to differ in several characters from *Ps. intybi* and *Ps. cichorii*. The specific identity of the organism is being studied.

Some species of Colletotrichum on leguminous forage crops. GILMAN, JOSEPH C., AND LOIS H. TIFFANY. Cultures of *Colletotrichum* isolated from soybeans (3), red clover (2), sweet clover (2), alfalfa (3), lima-beans (1), vetch (1), and peas (1) were compared with one another and with *C. graminicolum* and the Gloeosporium stage of *Glomerella cingulata*. Of the three cultures from soybeans, one is the conidial stage of *Glomerella glycines* (not *Colletotrichum glycines*), one that is similar to *Colletotrichum glycines* also resembles *C. truncatum*, and the third resembles *C. pisi*. On red clover *C. trifolii* and *C. destructivum* are good entities. On sweet clover one culture corresponds to *C. destructivum;* the other is similar to one of the alfalfa isolates, but as yet seems to be unnamed. From alfalfa, in addition to the culture similar to that on sweet clover, a second is like *C. destructivum* and the third, which has appeared in the literature as *C. graminicolum*, seems to be undescribed. The cultures from Lima bean, vetch, and peas were *C. truncatum*, *C. villosum*, and *C. pisi* respectively.

A biologic method for testing chemicals for the control of the potato ring-rot pathogen. GONZENBACH, CARLOS. Biologic tests of approximately 70 different chemicals were conducted to establish their relative bactericidal values upon *Corynebacterium sepedonicum* (Spieck and Kotth.) Skapt. and Burkh. The method used consisted of treating ring-rot-contaminated toothpicks with aqueous solutions of known and suspected bactericidal substances and introducing the toothpick into tomato plants. Inoculations made in young plants and near the growing point proved most reliable. The presence or absence of the pathogen was determined by the appearance or absence of characteristic and definite symptoms of the ring-rot disease or the recovery of the pathogen from the test plant. Synthetic dyes, chlorine derivatives, thio and dithiocarbamates, wetting agents, sulfa drugs, quaternary ammonium compounds, phenol and phenolic derivatives, mercury compounds, some fumigants, and a number of miscellaneous chemicals were tried. The mercury compounds as a group proved to be the most powerful disinfectants tested. Two mercury compounds, Mercury chloride and New Improved Semesan Bel, and the tetrachlorophenol Dowicide F were considered recommendable because of their germicidal effectiveness and other desirable characters.

Notes on the morphology of the genus Hemileia. GOPALKRISHNAN, K. S. In 32 species of *Hemileia* examined, three distinct types of sori, the Subepidermal, the Superstomal "A", and the Superstomal "B", are morphologically distinguished. In the Superstomal "B" type the number of feeder hyphae for the sorus is limited while in the Superstomal "A" type the feeder hpyhae are numerous and form fascicles in the substomal space. In *H. jasmini* the sorus arises out of a single hypha. All species studied were classified according to type of sorus and it was found that the Superstomal "A" sorus typified by *H. vastatrix* is dominant in the genus. It is suggested that on the basis of soral differences the genus may be subdivided although the validity of the existing species is not taken into consideration. Cross inoculations with various *Aecia*, occurring on a number of angiosperm hosts, on coffee leaves, and reciprocal inoculations of the basidiospores from *H. vastatrix* on the aecial hosts, are reported to be negative. Importance of primary sori in the classification of rusts is stressed and support is given to the view that basal cells and teliospore variations are not sure bases for generic distinction. The possible relationships of the genus, in the light of the knowledge of the sorus, to *Cystopsora* and *Gerwasia* are discussed.

Preliminary report on the pathology and parasitism of Physoderma graminis on Agropyron repens. GOPALKRISHNAN, K. S. *Physoderma graminis* (Busgen) Minden was first reported for the United States from Madison, Wisconsin, and was later collected at Ottawa, Canada, and in Illinois, Indiana, and Michigan. Leaves of *Agropyron repens* are infected in the embryonic stage and become thickened. Inflorescence is inhibited. The disease is apparent at first as yellow streaks which coalesce to form a patch throughout the length of the leaf. As the infection ages, the affected portions turn brown and

die. The hypertrophy observed is due to the enlargement of the cells harboring the resting sporangia. Germination of the resting sporangia, the ephemeral sporangia, and zoospores resembles that reported for *Physoderma zeae-maydis* on corn. Temperature is a critical factor in the germination of the resting sporangia. Cold treatment at 10° C. and burial in the soil for 2–3 weeks break the period of dormancy. Healthy plants of *Agropyron repens* growing at Notre Dame, Indiana, when transferred to infected soil (overwintered) from Madison, Wisconsin, became infected. One-year-old sporangial collections are still germinating in appreciable percentages. Preliminary cross inoculations with sporangial and zoospore suspensions on *Zea mays, Agrostis alba, Phleum pratense, Dactylis glomerata,* and *Euchlaena mexicana* were negative. The action of drugs on fresh resting sporangia is under investigation.

The role of antibiotics in soil. GOTTLIEB, DAVID, AND PAUL SIMINOFF. The presence of either *Streptomyces griseus* or *Aspergillus clavatus* in sterile soil inhibits the proliferation of *Bacillus subtilis.* With *S. griseus* this inhibition is not caused by the production of streptomycin, for even 500 μg per gm. of sterile soil will not prevent growth of the test organism. Streptomycin in high concentrations is inactivated in sandy, brown loam, and black clay loam soils from which it cannot be extracted by the usual chemical procedures. Adsorption by the clay in the soil accounts in part for this inactivation, as shown by the removal of this antibiotic from solution, the flocculation of the Wyoming bentonite, and its partial displacement by other basic compounds of high molecular weight such as methylene blue and Janus green. X-ray diffraction studies confirm the adsorption phenomenon. Furthermore, a nonstreptomycin-producing mutant of *S. griseus* inhibits growth of the test organism in soil. Other basic antibiotics are also adsorbed by clay. The acidic material, clavacin, on the other hand, is only partially inactivated in sterile soil and does not readily flocculate clay. The addition of more than 30 μg per gm. of soil prevents growth of *B. subtilis.* Preliminary experiments indicate that an eluate from soil containing *A. clavatus* possesses antibiotic activity.

A cucumber virus strain with a wide leguminous host range. HAGEDORN, D. J. During a 1948 study of viruses affecting canning peas in Wisconsin, an apparently new strain of cucumber virus 1 was isolated on two occasions from diseased pea plants found near Madison. Symptoms on artificially inoculated peas in the greenhouse included coarse leaf mottle, plant stunt, mild stem necrosis, plant wilt, and premature death. On cucumber, tobacco, and zinnia, symptoms typical of cucumber virus 1 appeared. Host range experiments indicated that the new cucumber strain was capable of infecting the following legumes: *Melilotus officinalis, Phaseolus vulgaris, Pisum sativum, Soja max, Trifolium hybridum, T. incarnatum, T. pratense, T. repens, Vicia faba, Vigna sinensis.* This appears to be a considerably wider leguminous host range than has been previously reported for the cucumber virus.

Physiologic specialization in Kabatiella caulivora. HANSON, E. W. A comparison of isolates of *Kabatiella caulivora* (Kirchn.) Karak. from the northern clover areas of the United States showed that this species is comprised of an indefinite number of physiologic races. All isolates used were started from single spores. On synthetic media, for example, isolates differed in color and topography of colonies, in ability to bud, in ability to produce mycelium, in tendency to sector, and in rate of growth. All of the isolates grew within the range of 4° to 28° C. but grew best at 20° to 24°. Two of the Wisconsin isolates grew approximately twice as fast at optimum temperatures as most other isolates. Wis.-1 differed from all others in that it grew well at 28°; the rate of growth of other isolates decreased rapidly when the temperature exceeded 24°. At least several races could be distinguished on the basis of pathogenicity tests on differential strains of red clover. For example, Dollard was resistant to Wis.-1 but susceptible to Wis.-2. Minnesota Sel. was moderately resistant to Ill.-1 but susceptible to Ill.-2.

Flue-cured tobacco resistant to mosaic and root rot. HENDERSON, R. G. Several lines of flue-cured tobacco resistant to mosaic and root rot have been developed by breeding. The mosaic-resistant parent was the *Nicotiana glutinosa-N. tabacum* hybrid obtained by Dr. F. O. Holmes, and the root-rot-resistant parent was a selection from a Turkish flue-cured cross. These mosaic-root-rot-resistant lines have been under yield and curing tests for two seasons. The advanced lines have been given the variety name Vamorr (Virginia-mosaic-root-rot-resistant). Two strains, Vamorr 48 and Vamorr 50, are being tested on a number of farms where mosaic has been a problem. They have had a complete resistance to mosaic and the field appearance of the plants is excellent. In preliminary tests, in the absence of mosaic in 1948, the yield of cured leaf of these

two strains was equal to that of the standard varieties, but quality of the leaf was slightly inferior. The degree of root-rot resistance is much higher than that of the Yellow Special and "400" varieties.

Pine gum flow following Fusarium inoculation. HEPTING, GEORGE H., AND RUSSELL B. CLAPPER. When spore suspensions of *Fusarium lateritium* f. *pini* were sprayed on standard turpentining streaks on pines, gum flow was prolonged beyond that following untreated chipping. Virginia pines chipped and inoculated once per season flowed gum all season, producing first-year average yields of about 2 lb. gum, the amount varying with tree size and tree-yield capacity. Inoculated streaks in slash pine chipped once the first 5 weeks, and again every 3 weeks the remainder of the year, and in longleaf pine once the first 3 weeks and then every 2 weeks, yielded gum volumes exceeding weekly untreated chipping for the first several weeks, and below standard chipping later in the season. The wood behind fungus-treated faces became pitch-soaked to depths exceeding those in untreated or acid chipping. Gum from fungus-treated trees had the chemical characteristics of gum from untreated trees, for the several constituents tested. The commercial possibilities of the fungus method are inconclusive to date.

Peach brown rot: Liquid lime sulphur and certain chlorine compounds as post-harvest dip treatments for control in the package. HEUBERGER, J. W., P. L. POULOS, AND J. J. HOOD. Employing the dip treatment developed in 1948, the relative effectiveness of liquid lime sulphur (4,2,1, qt.–100), liquid Chlorax (sodium chlorate; 500 to 4000 p.p.m. Cl), powdered Chlorax (sodium chlorate; 250 to 4000 p.p.m. Cl), Clorox (sodium hypochlorite; 500 to 4000 p.p.m. Cl), calcium chloride (0.25 to 1 per cent), and a slurry of nitrogen trichloride (100 to 2000 p.p.m. Cl) was determined. Tests were conducted on three varieties: Triogem, Halehaven, and Elberta. Data on rot control 4 days after treatment and packaging showed that liquid lime sulfur was the only chemical to give statistically significant control over the untreated. The chlorine compounds, in general, enhanced the color of the peaches but were injurious at high concentrations. Adding various chlorine compounds to liquid lime sulphur neither increased nor decreased control over that of liquid lime sulfur alone. Results with other chemicals showed that copper sulfate (0.5 to 125 p.p.m. Cu) gave no control, and that actidione (an antibiotic) used at 5 to 20 p.p.m. was very injurious.

Organic fungicides in the control of apple scab and European red mite. HILBORN, M. T., AND F. H. LATHROP. That elemental sulfur is an excellent fungicide for use on apples and that sulfur has insecticidal properties as well has long been known. There is some evidence that the current increase of European red mite in Maine may be attributed to the depletion of certain parasitic fauna by the increased use of sulfur. Two organic fungicides have shown promise not only in the control of scab but also in controlling European red mite. Some of the 341 compounds (mixed glyoxalidines), particularly 341-C, have satisfactorily controlled scab over a 3-year period and during this time no acaricide was necessary in these sprayed blocks. In adjoining blocks sprayed with elemental sulfur, 2 or 3 applications of an acaricide were necessary during each of the 3 years. The 341-B when formulated as a 10 per cent dust controlled apple scab but not European red mite. Apparently mite control was obtained through the lack of parasite depletion when the 341 compounds were used, since these materials have not been observed to have definite acaricidal properties. Arathane (CR 1639 or dinitro capryl phenyl crotonate) also has satisfactorily controlled apple scab and European red mite. Bioquin I, (copper 8-quinolinolate), Fermate (ferric dimethyl dithiocarbamate), and Phygon (2,3-dichloro—1,4-naphthoquinone) were tested for 1 year and while satisfactory for controlling scab did not control European red mite.

Some falcate-spored Colletotrichums on legumes. HOLDEMAN, Q. L. A number of falcate-spored *Colletotrichums* have been isolated from Lima bean, soybean, red clover, and *Lotus* spp. These isolates have been divided into two types on the basis of cultural characters on potato-dextrose agar, type 1 producing a white cottony growth with scattered sclerotium-like acervuli and relatively few conidia, and type 2 producing a strict growth and abundant conidia. Since cultures of type 2 have been obtained as variants from type 1, this difference does not seem significant. Greenhouse inoculations on pods of soybean and Lima bean with isolates from Lima bean, soybean, and red clover have resulted in similar symptoms. When seedlings of soybeans and Lima beans and plants of red clover were inoculated with these cultures, the Lima-bean culture was more pathogenic to Lima beans and the soybean culture to soybeans. The red-clover isolate was only weakly pathogenic. Another culture from Lima bean was only slightly pathogenic

to Lima bean or soybean. In field experiments these fungi spread from one host to the other. It is concluded that the isolates used, while differing in pathogenicity, are but strains of the same species, probably *Colletotrichum truncatum*.

Internal browning disease of tomato caused by strains of tobacco-mosaic virus. HOLMES, FRANCIS O. Outbreaks of internal browning disease of tomato in Middlesex County, New Jersey, showed close association between mosaic plantains (*Plantago* species) and first affected tomatoes. Both plantains and tomatoes were found to contain a series of similar but unusual strains of tobacco mosaic virus. This suggested that infection of the tomatoes had arisen from the diseased weeds. Experimental control was achieved by locating tomato fields at a distance from infected plantains and by use of mosaic-resistant lines of tomato.

Peach brown rot: Effect of low temperature storage for various periods on subsequent rot development in the package. HOOD, J. J., AND J. W. HEUBERGER. Two experiments were conducted on two varieties of peach in such manner as to permit statistical analysis of data. Three half-bushel tubs, commercially graded and packed, were used per treatment in each experiment. Five treatments were used on Halehaven variety: (1) room temperature (RT = approx. 85° F.), 4 days; (2) 40° F. 1 day, RT 4 days; (3) 40° F. 2 days, RT 4 days; (4) 40° F. 3 days, RT 4 days; and (5) 40° F. 4 days, RT 4 days. Percentage rot in each lot after 4 days at room temperature was 40, 50, 53, 55, and 49, respectively. Four treatments were used on Elberta: (1) 45° F. 4 days; (2) RT (approx. 75° F.) 4 days; (3) 45° F. 1 day, RT 4 days; and (4) 45° F. 2 days, RT 4 days. Percentage rot in the lot constantly at 45° F. and the other lots after 4 days at room temperature was 1, 32, 20, and 18, respectively. These data, on the whole, indicate that low-temperature storage has no pronounced effect on subsequent rotting at room temperatures. When data were based on number of days after harvest, less rot was present in the lots stored at low temperature for various periods; this was simply due to lack of rot development while at low temperatures.

Pathogenicity and occurrence in Wisconsin soils of Pythium species which cause seedling disease in corn. HOPPE, PAUL E., AND JOHN T. MIDDLETON. Seed decay in wounded corn kernels planted at low temperature in different Wisconsin soils ranged from none in scrub-oak forest soil to nearly 100 per cent in some cropped soils. A total of 138 *Pythium* isolates from decayed kernels removed from various soils were tested for pathogenicity in artificial inoculations. The average pre-emergence death in the non-inoculated controls and that due to the species identified among 58 *Pythium* isolates was: Controls—6 per cent; *P. vexans*—20 per cent; *P. paroecandrum*—53 per cent; *P. rostratum*—59 per cent; *P. ultimum*—62 per cent (variability within species); *P. debaryanum*—87 per cent; and *P. irregulare*—89 per cent. The source and frequency of the identified cultures was as follows: Plainfield sand (oats preceding crop)—13 *P. irregulare*, 1 *P. rostratum;* Plainfield sand (alfalfa preceding)—5 *P. irregulare*, 2 *P. ultimum;* peat (corn preceding)—6 *P. ultimum*, 4 *P. debaryanum;* Miami silt loam (corn preceding)—4 *P. ultimum*, 2 *P. vexans;* Dodgeville silt loam (corn preceding)—20 *P. ultimum;* Spencer silt loam (alfalfa preceding)—2 *P. paroecandrum*, 1 *P. irregulare*, 1 *P. debaryanum*. The extremely pathogenic isolates, including the 80 yet unidentified, usually were recovered from the soils in which seed decay had been most severe, whereas isolates from soils having a lower level of disease generally proved less virulent. Presumably the pathogens chiefly responsible for the disease were the ones consistently isolated. It is concluded that certain *Pythium* species are associated with soil type but the association probably is influenced by crop sequence.

Protective action of bis (2-hydroxy, 5-chlorophenyl) sulfide. HORSFALL, J. G., AND SAUL RICH. In 1943, bis (2-hydroxy, 5-chlorophenyl) sulfide (Cr-305) was tested as a fungicide in the laboratory and proved to have high tenacity. It was toxic to *Sclerotinia fructicola* but not to *Stemphyllium sarcinaeformae*. Cr-305 turned out to be as specific in its field performance as it was in the laboratory. Thus it gave excellent control of apple scab and rose black spot, but failed to control celery leaf spots, bean anthracnose, potato late blight, and tomato early blight. Cr-305 is of interest as an apple fungicide with a long residual effect because of its high tenacity. On apple foliage, it is also able to eradicate young scab infections. In 1949, the experimental formulations of Cr-305 caused russeting of apple fruit. Whether the russeting would be caused by pure Cr-305 is not known. Foliage injury has so far been negligible. Some 50 analogues of Cr-305 are currently on trial.

A laboratory technique for testing cold resistance in oats. IVANOFF, S. S. A new approach toward determining cold resistance in plants has been made which promises to

hasten the breeding of winter-hardy oats. It is strictly a laboratory procedure, and employs activated seeds rather than seedlings or more mature plants. Several hundred thousand seeds may be conveniently and quickly tested at one time, with relatively little labor, skill, or equipment. The method is based on the finding that when seeds of winter-hardy, semi-winter-hardy, and non-winter-hardy oats were soaked in running water for 20 hr. at 25° C., and then frozen at −8° C., the degree of survival of the three oat types corresponded to their relative winter-hardiness as known from practical field experience. Other factors beside inherent cold hardiness determined the degree of survival: temperature of running water, length of soaking, and degree of freezing. By lowering the freezing temperature progressively from −8° C. to −28° C., increasingly greater kill was effected until none or only a few seeds per hundred thousand of the most winter-hardy oats remained germinable. After testing several million seeds, the survivors were grown to maturity for increase and further testing.

Influence of nitrogen nutrition on the development of bacterial wilt in tomato and tobacco. KELMAN, ARTHUR. The influence of the nitrogen nutrition of Variety 402 tobacco and Marglobe tomato plants on the development of bacterial wilt (*Pseudomonas solanacearum* E. F. S.) was studied in sand cultures supplied with nutrient solutions by sub-irrigation. After inoculation of cut roots with standardized bacterial suspensions, disease development in tobacco was usually most rapid at nitrogen levels most favorable for plant growth. High levels of nitrogen above the optimum for growth significantly depressed wilt development. In an experiment with tomato using two different nitrogen sources, $Ca(NO_3)_2$ and NH_4NO_3, at low, medium, and high levels, those plants supplied with NH_4NO_3 wilted most rapidly. There were no marked differences in rate of wilting at the various levels of NH_4NO_3. However, in the $Ca(NO_3)_2$ series disease development was significantly depressed at the highest concentration (896 p.p.m. N). In another test tomato plants were supplied with solutions containing NO_3-N alone and combinations of NH_4- and NO_3-N in ratios of 1:1 and 3:1 at two concentrations (224 and 448 p.p.m. N). Wilt development was most rapid in plants supplied with the high ratio of NH_4-N, and least rapid in the series supplied with NO_3-N alone.

Temperature and the development of Rhizoctonia solani on legumes. KHAN, A. M. Pathogenicity of two races, CT1C and CP1, of *Rhizoctonia solani* on two legumes, soybean *(Soya max)* and pea *(Pisum sativum)*, varied with soil temperature and with host. Race CT1C, originally isolated from soybean, killed 40 per cent of the soybean and pea plants at low (14°–17° C.) and high (28°–32° C.) temperatures, while at intermediate temperatures only 20–30 per cent of the plants were killed. Virulence of race CP1, originally isolated from flax, was similar to that of race CT1C when the soybean host was used. On the pea host, on the other hand, CP1 killed 18 per cent of the plants at low temperatures and the mortality increased gradually with temperature until finally at high temperatures it was 40 per cent. Similar results were obtained in field tests. CT1C and CP1 have an optimum growth in culture at 24° C. and 18° C. respectively. Thus, there seems to be no correlation between pathogenicity on these legumes and optimum temperatures for growth in culture.

Root and crown rots of red clover in Wisconsin. KILPATRICK, R. A., AND E. W. HANSON. Root rots, crown rots, and wilt rank among the most important diseases of red clover in Wisconsin. Preliminary studies indicate that they are caused by a complex of fungi including *Fusarium oxysporum, F. solani, F. moniliforme, F. roseum, Gleocladium* sp., and *Pythium* sp. *Rhizoctonia* spp. and an unidentified fungus very similar to *Phoma* have been among the other fungi commonly isolated but not yet tested for pathogenicity. First-year as well as second-year stands may undergo substantial injury. In a naturally infested field, 10 strains of red clover (4 replications) were sown April 26, 1949. Stand counts were made May 6 and again August 31; during this interval of 4 months the losses in stand ranged from 39 per cent in Dollard to 52 per cent in Emerson, while the average decrease for all strains was 45 per cent. Most plants showed infection 2 months after seeding and the severity of the rots increased as the summer progressed. More than half of 2500 isolates obtained from plants grown in this field were *F. oxysporum*. The relative prevalence and severity of the fungi concerned are influenced by the age of the host, climatic conditions, cultural practices, the presence of insects, and other factors.

Sprays to control chlorosis in flax and strawberries grown on alkaline soil in Minnesota. KING, T. H., MARTIN TVEIT, AND A. DAVID BASKIN. Chlorosis occurs commonly on many types of plants in the upper Red River Valley of Minnesota, and attempts to correct it by application of fertilizers and soil amendments have failed. Preliminary results indicate that spray solutions containing iron correct chlorosis in plants grown in Red River Valley soils. Correction was not complete when nutrient solutions were added to soil

at rates 20 times that of spray applications. Neither applications of sulphur to soil at rates up to 5 tons an acre, nor of sulphuric acid, corrected chlorosis in the greenhouse. Spraying Sheyenne flax with solutions containing iron corrected chlorosis both in greenhouse and field, and apparently increased flower formation and boll set in the greenhouse. Yields of Dakota flax sprayed in the field were significantly increased. Chlorosis of strawberries was also corrected in both greenhouse and field tests with iron-containing sprays. Ferric dimethyl dithiocarbamate (Fermate) at the rate of 8 lb. per 100 gal. water (approximately 900 p.p.m. iron) and iron sulphate, $Fe_2(SO_4)_3$, at 2000 and 4000 p.p.m. were used as sprays.

Studies on Helminthosporium carbonum Ullstrup on corn. KINGSOLVER, C. H., AND C. T. YUAN. In 1946 plants of the inbred Mo21a corn were severely attacked by *Helminthosporium carbonum*. Study during three seasons revealed the presence of this fungus on Mo21a, NC37, K61, K44, H7, K27, K150, and some lines in process of inbreeding. *H. carbonum* was observed to attack susceptible hosts from the time the seed coat ruptured until the plant was mature. On leaves, the symptoms were those previously described by Ullstrup as characteristic of race I of this fungus. Thirty-one isolates were studied. Average conidial measurements of 62.4 $\mu \times 13.2$ μ and an average of 6.5 septa were in agreement with published figures. Source or host passage did not affect conidial size or pathogenicity. Growth on media was optimum near 25° C., good from 20° to 30° C., and maximum near 35° C. Infection was obtained by hypodermic inoculation of plants, by atomizing plants, and through soil and seed infestation. Spread from infected to healthy plants was obtained by use of air currents. Severity of disease development seemed primarily dependent upon humidity. Greenhouse studies on seedling plants agreed with field observations, except that foliar symptoms could be produced in the greenhouse on material which in the field had symptoms only on seed.

Etiological association of a Brevipalpus mite with Florida scaly bark of citrus. KNORR, L. CARL. Numerous explanations concerning the cause of Florida scaly bark of citrus have been made during the century-long history of this disease. While various fungi, a bacterium, and a virus have been suggested or implicated in the past, it is the purpose here to report that yet a different etiological agent is involved. An arachnid was isolated from infected orange twigs and cultured on sweet orange seedlings. Four months later leaf spots appeared reproducing in their morphology, ontogeny, and marginal placement those of the Florida scaly bark disease. Bark cankers also resembled those of the same disease in their form and occurrence on nearly matured wood. The mite has been identified as a species of *Brevipalpus* (syn. *Tenuipalpus*). Concurrence of this etiological agent with that shown to be associated with lepra in Argentina lends further evidence to the synonymity of Florida scaly bark or nailhead rust and South American lepra explosiva, both diseases being now justifiably joined by the common name "leprosis."

Relation of mechanical injury and seed treatment to germination of flax seed of the 1948 crop. KOMMEDAHL, T., AND J. J. CHRISTENSEN. Many lots of flax seed produced in Minnesota in 1948 germinated very poorly in soil, some lots less than 20 per cent, chiefly because of mechanical injuries during harvest. All of the 120 seed lots examined had cracked or chipped seed coats, with a maximum of 46 per cent in one lot; in addition there were varying degrees of internal injuries not apparent until the seeds germinated. Injured seed usually produced abnormal plants; in some lots more than 40 per cent of the seedlings were abnormal. Many types of root injuries occurred; frequently the root cap or tip was broken off, sometimes it was split or cracked, occasionally it was crushed, and roots often were swollen and contorted. One or both cotyledons frequently were cracked or injured in various other ways. Many seed lots that produced poor stands in soil germinated well on blotters: in one lot of Minerva, for example, the respective stands were 21 and 88 per cent. Seed treatment of 120 seed lots with organic mercury dusts greatly increased stands, in some cases more than 100 per cent.

Prevalence of Colletotrichum linicolum on flax seed and seedling infection of flax varieties. KOMMEDAHL, T., J. J. CHRISTENSEN, AND E. SCHWINGHAMER. *Colletotrichum linicolum* is frequently seed-borne on flax in Minnesota. Depending on the seed source, year produced, and the variety, the percentage of infection ranges from 0 to 60. Infected seed usually produces blighted seedlings unless seed is treated with a fungicide. Tests of 645 hybrid lines with naturally infected seed planted in steamed soil in the greenhouse indicated marked differences in susceptibility. Seeds of flax varieties to be tested were placed in folded strips (1 in. deep) of wet blotting paper. Three-day-old seedlings within the folded paper were dipped in a spore suspension of *C. linicolum* and planted immediately in soil. Ten days later the folded blotters containing seedlings were removed from

the soil and infected seedlings were counted. Of 20 varieties tested, Minerva, Crystal, Rocket, and Arrow were the most resistant, while Bison, Dakota, and Punjab were completely susceptible. Merits of this technic include: constant contact of inoculum with seedlings, thus facilitating infection; absence of soil from roots; uniformity of varietal testing; simplicity of operation; ease of note-taking; economy of space; and a 12- to 14-day testing period.

Longevity of inoculum of Sclerotinia trifoliorum prepared from cultures grown on grain. KREITLOW, K. W. Large quantities of inoculum of *Sclerotinia trifoliorum* have been required for field and greenhouse tests with forage legumes at the Pasture Laboratory. Preliminary investigation demonstrated that dried grain inoculum offered certain advantages over other kinds tested, especially in ease of application, preparation and storage of large amounts at the convenience of the investigator, and availability when plants are at the proper stage for testing. Inoculum was prepared by growing pathogenic isolates on a steamed mixture of 2 parts wheat and 1 part oats in 250-ml. Erlenmeyer flasks for 3 weeks at 15° C. The grain inoculum was then dried at room temperature, ground (no finer than 40-mesh), and stored at 5° C. Tests on samples from 10 batches of inoculum demonstrated that the dried grain inoculum could be stored at 5° C. for at least 16 months without loss of virulence.

Role of Fusarium in loss of red clover stands. KREITLOW, K. W., AND R. G. HANSON. Stands of red clover in Pennsylvania may be severely depleted during the second year. The plants die from invasion by fungi that enter the host principally through wounds in the crown and tap root. Two species of *Fusarium, F. solani* and *F. oxysporum,* were consistently isolated from diseased plants. Pathogenicity of the Fusarium species to red clover was demonstrated by placing inoculum in contact with scarified or pruned tap roots of young plants transplanted to flats of steamed soil. Best infection was obtained if soil temperature was above 30° C. Pathogenicity of Fusarium isolates from red clover was also tested on 18 species of *Trifolium,* 5 species of *Medicago,* and 4 species of *Melilotus.* Only *T. repens, T. fragiferum,* and *Melilotus suaveolens* resisted infection.

Chlorobromopropene, a potential fungicidal soil fumigant. KREUTZER, W. A., AND J. TH. W. MONTAGNE. In screening studies in which numerous materials were tested for efficacy as fungicidal soil fumigants, a promising material, chlorobromopropene (1-chloro-3-bromopropene-1) was found. In soil, in closed and in open containers, the fumigant was fungicidal in 48 hr. to *Fusarium solani, Verticillium albo-atrum,* and dried sclerotia of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* at 0.02–0.04 ml. per liter and 0.025–0.075 ml. per liter, respectively. For field trials, a technical product containing 65 per cent by weight of the active material was used. An application by injection of 25 gal. per acre 3 weeks prior to planting gave economic control of pink root of onions (*Pyrenochaeta terrestris*). The yield of onions from treated plots was approximately four times that from control plots. Control of pre-emergence damping-off of peas (*Pythium ultimum*) and of a replant disease of peach seedlings was obtained following injection rates of 10 and 30 gal. per acre respectively. No soil seal was utilized in these field studies. Soil temperatures and moistures most favorable for fungicidal effectiveness were 50°–70° F. and below 85 per cent of the moisture equivalent. No phytotoxicity was noted provided a 2- to 3-week interval was observed between treating and planting.

Asclepias yellows. KUNKEL, L. O. A yellows-type disease on *Asclepias syriaca* L., growing in the vicinity of Ithaca, New York, near an orchard in which X-disease of peach occurred, was suspected of being identical with the latter. Transmission to carrot, tomato, and *Vinca rosea* L. was accomplished by the dodder method using *Cuscuta campestris* Yuncker. The disease was readily transmitted in *Vinca rosea* by grafting. All attempts to transmit it to carrots, tomatoes, and periwinkles manually by means of juice from diseased tissues failed. A comparison of the symptoms produced in the plants to which it was taken with those caused by X-disease of peach in the same species showed that the two maladies were distinct. A comparison with aster yellows, peach yellows, little peach, and peach rosette in one or more mutual host plants showed that it also differed from each of these. Asclepias yellows has not been identified with any yellows previously described.

Root grafts as a possible means for local transmission of oak wilt. KUNTZ, J. E., AND A. J. RIKER. The progressive spread of oak wilt in local areas has been studied more intensively than the spread over long distances, because of the greater feasibility of local control. Periodic examinations of 36 oak-wilt plots, established in central and southern Wisconsin from 1939 to 1947, have demonstrated such tree-to-tree spread. An

additional 47 control plots included: (1) eradication of dead and wilting trees, (2) periodic application of DDT sprays, (3) combinations of these, (4) various disposition of diseased material, and (5) poisoning trees. Items (1) through (4) had little effect on local spread. Abundant and widespread natural grafting of black oak roots was revealed by digging and washing out of root systems, and by the movement of poisons between trees—observed up to a distance of 28 feet. Dyes and spores of the oak-wilt fungus also readily passed through such grafts. *Chalara quercina* H. was isolated on the symptomless side of root grafts between wilted and "healthy" trees. Bur oak roots were found to graft with one another but not onto black oaks. Thus, adjacent oaks may have united root systems. Interruptions of these systems on six plots in 1947 by poisoning adjacent healthy trees have thus far given local control.

Efficacy of dichlorobutene as a soil fumigant against Heterodera rostochiensis Wollenweber and Heterodera marioni (Cornu) Goodey. LEAR, BERT. On the basis of data obtained from exploratory greenhouse crock tests and small field plots, dichlorobutene appeared to be a very effective soil fumigant against both the golden and root knot nematodes. Counts of larvae emerging from cysts screened from soil previously treated in glazed gallon crocks showed that 0.5 ml. of either the crude or purified material eliminated the pest and half that dosage gave excellent control. In field plots, injections of 3.2 ml. made 4 in. deep on 10-in. centers reduced the viable nematode population 91 per cent and increased yields of Green Mountain potatoes 11.7 times. Gall counts on roots of squash plants were used to compare efficacies against root-knot nematode. A mean of 656 galls per root for a 5-week-old squash plant was obtained from check plants whereas no galls were found on plants growing in crocks of soil treated with 0.5 ml. of dichlorobutene. When equal dosages of dichlorobutene and DD (dichloropropylene and dichloropropane) were injected in the soil, toxic amounts of DD diffused farther through the soil laterally and vertically. Dichlorobutene also tended to remain in the soil for longer periods.

Use of methyl bromide for killing golden nematode cysts on used bags and in soil samples. LEAR, BERT, AND W. F. MAI. Complete eradication of living cysts of the golden nematode adhering to used burlap bags has been obtained from fumigation with methyl bromide. No viable cysts have been recovered from bags receiving an exposure of 16 hr. at a dosage of 6 ml. per cu. ft. or for 2 hr. at 12 ml. per cu. ft. of space. Three types of fumigation enclosures were used, a 55-gal. metal drum, beneath a gas-proof tarpaulin, and a copper lined fumigation chamber. As many as 600 bags tied in bundles of 50 have been fumigated successfully. Cysts imbedded in triturated tubers or in wet and dry soil have been successfully killed in various parts of the enclosures. Using the same dosages of methyl bromide all cysts in soil samples as large as 10 lb. have been killed by placing the samples in the enclosure with the top of the soil container, usually a paper bag, open. All tests were made at temperatures above 65° F.

Bioassay of tetramethylthiuramdisulfide. LEBEN, CURT, AND G. W. KEITT. An agar plate assay has been devised for the determination of micro amounts of tetramethylthiuramdisulfide (TMTD) or Arasan SF, which contains TMTD as the active ingredient. Blotting paper disks, containing solutions of the fungicide in ethanol, were placed on agar (gm. per liter: peptone, 5; agar, 17) seeded with the assay fungus, *Glomerella cingulata.* A standard consisting of a series of known concentrations of TMTD was tested each time the assay was set up. After 24–30 hr. at 28° C., the size of the inhibition zones was measured. A standard curve was plotted with the diameter of the inhibition zone and the log of the TMTD concentration as coordinates. A straight line dosage-response curve was observed over the range of 2.5 to 20 μg. per ml. The TMTD concentration of solutions of unknown TMTD content was determined by interpolation of this curve. The amount of Arasan SF on corn seed has been determined by assaying the ethanol extract of the treated seed.

Inheritance of resistance to bacterial canker in cowpea, Vigna sinensis. LEFEBVRE, C. L., AND HELEN S. SHERWIN. Cowpea varieties that had previously been determined to be homozygous for resistance to the bacterial canker organism, *Xanthomonas vignicola,* were crossed with susceptible varieties in the greenhouse. The progenies were inoculated by stabbing the stem twice just below the primary leaves with an arrow-point needle covered with bacteria. All inoculations were made when the first trifoliolate leaves were beginning to unfold. The first generation was resistant in all crosses. In the F_2 generation, however, segregation appeared to fit a 15: 1 ratio more closely than any other, but when the F_3 lines were inoculated it was found that 15 to 20 per cent were completely susceptible, indicating that these plants escaped infection in the F_2. When this difference is taken into account and a correction made, then a close fit to a 3: 1 ratio is ob-

tained. A study of the progenies from 10 crosses suggests, therefore, that a single pair of factors is responsible for resistance.

Survival of races of Puccinia graminis tritici on susceptible wheats. LOEGERING, W. Q. In the greenhouse, mixtures of races 17 and 19 of *Puccinia graminis tritici* were cultured for six to seven generations on Mindum durum and Little Club and Fulcaster wheats; and mixtures of races 17 and 56 were cultured for the same number of generations on Ceres, Little Club, and Fulcaster wheats. There appeared to be no differences in susceptibility of any of these varieties to the two races in the respective mixtures. Nevertheless race 17 survived better in each mixture, but there were pronounced and consistent differences in its survival on the three hosts. The poorest survival of race 17 in mixtures with race 19 was on Mindum durum, and in mixtures with race 56 its poorest survival was on Ceres wheat. In general, the trends agreed with field observations with respect to prevalence of these races on certain varieties, although race 17 survived better in the greenhouse than was expected from field observations. It appears, therefore, that multiplication and survival of physiologic races may differ in different mixtures and may vary with the variety on which the mixtures develop.

Stimulation of golden nematode larvae by root leachings. LOWNSBERY, B. F. Response of encysted golden nematode (*Heterodera rostochiensis*) larvae to the stimulus of leachings from potted potato plants provides a rapid, objective method for assaying viability of these larvae. The accuracy and rapidity of this assay have been improved and its limitations more clearly defined. A method was developed for extracting cysts from infested soil at the rate of approximately 6000 per min. using a simple flotation and sieving device. It was possible to obtain batches of like numbers of cysts within a 6 per cent error by weighing the material obtained by flotation and sieving on a torsion balance. The stimulant was obtained from both roots and tops of the potato plant, but was most easily obtained by leaching the potted plants. Leachings obtained after as much as 4 liters dripped from a single plant remained effective. Potency of the leachings increased with size of root system but was not correlated with age of plant. Emigration of larvae from cysts into the leachings occurred between 15° and 30° C. with a maximum near 21° C., and was inhibited by light.

Studies in Septoria lycopersici Speg. MACNEILL, BLAIR H. The initiation of parasitic activity is dependent upon a minimum period of 48 hr. at saturation for the promotion of germination and stomatal penetration. Nutrition and light predispose the host to disease, while temperature acting subsequent to penetration may interrupt the developmental rhythm of the fungus to delay sporulation. The resulting plasticity of symptom expression, as conditioned by these factors modifying host-parasite interaction, does not allow for the use of lesion size alone as a measure of host resistance. The behavior of spores germinating on leaves of various hosts suggests a prepenetration phase of host-parasite interaction. Leaf colonization is intercellular, and while the presence of haustoria was not demonstrated there is a very intimate association between fungus and the tissue parasitized. The equilibrium maintained between compatible host and parasite is displaced upon completion of the fungus fruiting cycle, and no further spread of the colony involved can occur. The Feulgen stain, modified to suit the type of material at hand, indicates a uninucleate condition of both spore and mycelial cells. There is no suggestion of a sexual stage. The existence of quantitative and qualitative differences in pathogenicity of at least two strains of the fungus has been demonstrated.

Organic and chromate compounds as seed treatments. MCCALLAN, S. E. A. Employing the pea seed test, 597 organic or chromate compounds were evaluated as seed protectants. The outstanding 11 were then tested on Lima bean, beets, cucurbits, sweet corn, cabbage, and spinach. The most promising were: No. 201, chloroacetoacetanilide; 854, copper chloroacetoacetanilide; 5400, reaction product of dimethyldithiocarbamate and sulfur dichloride; L224, mercury zinc chromate; and L640, copper zinc chromate. These five were compared to Arasan (tetramethyl-thiuram-disulfide), Semesan (hydroxymercurichlorophenol), Spergon (tetrachloro-parabenzoquinone), Phygon (2,3,dichlor-1,4-naphthoquinone), and Yellow Cuprocide (yellow cuprous oxide) in field tests at Yonkers, N. Y., and Seabrook, N. J., on 10 crops, *i.e.*, the above plus tomato, snap bean, and peanut. There was high correlation between the field and greenhouse tests for many crops. Good results were obtained as follows: No. 201—pea, spinach, and muskmelon; 854—pea, cabbage, spinach, cucurbits, and Lima bean; 5400—cabbage, spinach, Lima bean, and cucurbits; L224—corn, cabbage, and pea; L640—pea. Lima beans are injured by L224 and 201.

Factors influencing glyoxalidine spray injury to potted apple trees. MCCALLAN, S. E. A. The factors influencing glyoxalidine spray injury to potted dwarf McIntosh apple trees were studied in laboratory and greenhouse. Typical early-season bronzing of the lower surface of new leaves was induced. Injury increases rapidly with temperature and is pronounced at 90° F. and above. Four hours' exposure at 90° F. will produce symptoms which are not greatly increased by longer exposure. A humid atmosphere is more conducive to injury than a dry one (50 per cent relative humidity). The addition of lime to the 341C formulation does not modify the injury, but with the 341SC formulation (made from 98 per cent pure stearic acid), marked reduction occurs. The 341SC formulation appears several-fold less injurious than the 341C formulation.

Plant growth and Fusarium spore germination in greenhouse beds subirrigated with nutrient solutions containing fungicides. MCCLELLAN, W. D. Gladiolus, tomatoes, Easter lilies, snapdragons, annual stocks, carnations, geraniums, and China asters were grown in 32 subirrigated beds containing Haydite and watered twice daily with 200 l. of nutrient solution. There were a check and 15 fungicide treatments including one or two concentrations of Fermate (ferric dimethyl dithiocarbamate), Tersan (tetramethyl thiuram disulfide), Dowicide B (sodium trichlorophenate), G-4(2.2′ methylenebis [4-chlorophenol]), Mersolite 8 (phenyl mercuric acetate), Roccal (alkyl dimethyl benzyl ammonium chloride), copper sulfate, 8-hydroxyquinoline sulfate, and 8-hydroxyquinoline benzoate. All treatments were added to the nutrient solutions except one Fermate treatment which was mixed with the Haydite. The fungicides affected plant growth more in B grade Haydite than in the FF grade. Growth was seriously depressed by Fermate, and Roccal caused severe injury. Poor growth of gladiolus occurred with Dowicide B, of lilies with Mersolite 8, of geraniums with 8-hydroxyquinoline sulfate, Dowicide B, G-4, and Mersolite 8, and of snapdragons with 8-hydroxyquinoline sulfate, 8-hydroxyquinoline benzoate, Dowicide B, G-4, and Mersolite 8. Growth of all plants was good with Tersan. Germination of macroconidia of *Fusarium oxysporum* f. *callistephi* was tested by suspending conidia in agar in cheese cloth bags in the nutrient solutions or burying them in the Haydite for 24 hr. Germination in the nutrient solution was prevented by all but Dowicide B (5 p.p.m.) and copper sulfate, but only by Fermate, 8-hydroxyquinoline benzoate, Dowicide B (20 p.p.m.), and Mersolite 8 when the macroconidia were buried in the Haydite.

Effect of high soil populations of the golden nematode, Heterodera rostochiensis Wollenweber, on the yields of Irish Cobbler and Green Mountain potatoes. MAI, W. F. In the fall of 1947 and again in the fall of 1948 part of an area of heavily-infested soil received two 250-lb. applications of Shell DD (dichloropropylene and dichloropropane). In the spring of 1948 there were 19.5 viable cysts per oz. of soil in the untreated area and 0.04 in the treated soil. The corresponding figures for the spring of 1949 were 17.3 and 0.27. During 1948 and 1949 comparative yields of Irish Cobbler, an early variety, and Green Mountain, a late variety, were obtained on the treated and untreated areas. The Cobblers yielded 60 per cent less weight of U. S. No. 1 tubers on the heavily-infested soil in 1948 and 68 per cent less in 1949. The corresponding percentage reductions for the Green Mountain variety were 84 and 72. In 1948 the Green Mountain variety significantly outyielded the Cobbler variety on the treated area while yields of the Cobbler variety were significantly greater on the untreated soil. In 1949 there were no significant yield differences between the two varieties on either area. The 1948 growing season was ideal for the growth of potatoes while the lack of rainfall markedly reduced yields in both plots during 1949.

The effect of latent viruses on the growth of cherry trees. MILBRATH, J. A. Shirofugen and Kwanzan flowering cherry have been found to be reliable index plants for all known strains of cherry ring spot and cherry latent viruses. When nursery trees were propagated in adjacent rows the stock from the virus-free trees was taller and sturdier than similar trees from mother stocks which reacted on Shirofugen and Kwanzan. On adjacent rows, measurements based on 200 trees, virus-free stock averaged 12 or more inches taller than stock infected with latent viruses. Stand counts showed 25 to 50 per cent more trees in the virus-free rows. Most sweet and sour cherries from many different localities carry one or more viruses in a latent condition.

The Western X-disease of peach and the little-cherry complex in Oregon. MILBRATH, J. A. Western X-disease of peach and little cherry as they occur in Oregon show a close relationship. They often occur in mixed plantings in the same orchard. Western X-disease symptoms on peach have been produced by inoculations with little-cherry inocu-

lum from both sweet and sour cherry. Many peach trees are destroyed each year because of X-disease and the rate of spread of little cherry is rapid. Cherry orchards with one per cent of the trees infected with little cherry often have 2 per cent of the trees infected the next year, those with 10 per cent infection may have 20 per cent the next year, and those with 25 per cent may have 50 per cent of the trees infected in one year's time. This demonstrates the serious nature of the spread of this disease. Albino, a similar disease which shows no Western X-disease relationship, spreads even faster, and destroys the trees in a shorter time. No practical method of control is known for either Western X-disease or little cherry and eradication is impossible.

Control of sour cherry curl leaf. MOORE, J. DUAIN, A. R. ALBERT, and J. G. MOORE. In the fall of 1947 an experiment was begun to determine the effects of mulch and fertilizer in the control of curl leaf of sour cherry. A Montmorency orchard planted in 1942 was selected for these studies. The trees in this orchard had made little terminal growth for several years and many were dying. All trees bore numerous leaves that curled upward, and many curled leaves had marginal necrosis. The summer of 1948 was unusually dry, and substantial recovery occurred only in plots that received straw mulch, either alone or with 0–9–27 fertilizer. By August, 1949, however, striking differences among the various treatments were obtained. The best recovery resulted with and without fertilizer in the mulched plots, but significant response to treatment occurred, also without mulch, in plots receiving 0–9–27, 0–0–27, or 0–9–27 with $MnSO_4$. Trees that received 0–9–0 fertilizer were still as bad as those that received no treatment. Improvement is attributed to increase of available potassium.

Relations of spray programs to processing sour cherries. MOORE, J. DUAIN, and G. W. KEITT. Montmorency cherry fruit size was markedly influenced by spray materials and programs used for cherry leaf-spot control in Wisconsin in 1949. Studies were begun to determine whether the processing of the fruits might also be affected. In the experimental work various programs of Bordeaux mixture, "insoluble" coppers, and organic fungicides were used. There were six replicated single-tree plots of each of 14 spray programs. From each tree, an 8-lb. sample of cherries was cold packed, and four No. 2 cans were hot packed. In general the smallest fruit sizes, the highest Brix test, and the highest drained weights were obtained with cherries from trees that received copper as the fungicide; and the largest fruit sizes, the lowest Brix tests, and the lowest drained weights were obtained with cherries sprayed with an organic fungicide. The use of Dowax 222 with Tennessee "34" (34 per cent metallic copper) in all applications before harvest occasioned large fruits with the lowest Brix tests and the lowest drained weights. There were highly significant differences in fruit sizes and Brix tests among the various spray programs, but the drained weights were not significantly different. The drained weights required to meet government standards were obtained with fruits from all plots.

Development of varieties of spearmint resistant to Verticillium wilt and to rust. NELSON, RAY. The Highland variety of spearmint (*Mentha spicata*) is susceptible to the destructive Verticillium wilt disease in Michigan and is also very susceptible to rust (*Puccinia menthae*). The American variety is more resistant to wilt but is equally susceptible to rust. These diseases are destructive to Michigan plantings when seasonal conditions are favorable. In *Mentha crispa*, which yields spearmint oil, wilt resistance is apparently conditioned by two complementary dominant genes and rust resistance by a single gene. The commercial spearmints are nearly self-sterile, but *Mentha crispa* is fertile and produces pollen freely. In 1947, mass hybridizations of the Highland and American varieties of *Mentha spicata* with *Mentha crispa* yielded 27,000 seeds from the Highland and 8,000 from the American, approximately 10 per cent of which germinated. The Highland × *M. crispa* seedlings were superior and from them plants were selected for resistance to wilt and rust. They were propagated in the greenhouse and tested in Verticillium-infested soil in 1949 for yield and resistance. With soil temperatures and moisture optimum for wilt, some of the selections were highly resistant to wilt and rust and yielded larger quantities of oil than the seed parent. Oil qualities appear satisfactory in preliminary tests.

Transmission of corn stunt in Mexico by a new insect vector, Baldulus elimatus. NIEDERHAUSER, JOHN S., AND JAVIER CERVANTES. In greenhouse experiments the corn stunt virus was transmitted by the leafhopper *Baldulus elimatus* Ball. The minimum incubation period (of the virus in the insect) that has been observed is 20 days. On inoculated plants the earliest detectable symptoms appeared 4–7 weeks after inoculation.

The relative abundance of *B. elimatus* in corn fields in the Mesa Central of Mexico indicates that this insect is probably responsible for much of the field spread of corn stunt in this part of Mexico.

Manganese content of apple trees. ORTON, C. R., AND GENEVIEVE CLULO. Internal bark necrosis of apple has been shown by Berg and Clulo to be caused by excessive intake of manganese. Manganese analyses of leaves and bark of individual trees show great variation in manganese absorption from soil of uniform manganese content. Likewise there is a marked difference among apple varieties in their ability to absorb manganese as well as their ability to tolerate excessive amounts of Mn. Analysis of bark of 150 trees as received from the nursery varied from 29 to 234 p.p.m. Mn and averaged 83.5 p.p.m. During three successive years the Mn content of current-season bark of 46 Delicious trees grown in uniform manganiferous soil varied from 144 to 535 (average 292) p.p.m. without showing internal bark necrosis. Fifteen Delicious trees which developed internal bark necrosis under the same conditions showed variations in Mn content from 372 to 798 (average 495) p.p.m. Nine other varieties of apples grown under the same conditions showed average variations in Mn content of bark from 135 in Grimes to 819 p.p.m. in Stayman without showing internal bark necrosis. The Mn content of leaves is much higher than that of bark, but shows similar variations between individual trees.

The proteins of green leaves. V. Serological relationships between tobacco mosaic virus and the main nucleoprotein component in tobacco leaf cytoplasm. OWEN, RAY D., AND S. G. WILDMAN. The component comprising the greater part of the cytoplasmic proteins of healthy tobacco leaves, when injected into rabbits and chickens, induces the formation of high-titer, specific antisera. Presumably because of its purified, concentrated, and undenatured character, this protein is a more effective antigen than were the leaf juices used for previous comparable studies. Antisera to the normal protein cross-react vigorously with purified preparations of tobacco mosaic virus (TMV) protein. Quantitative precipitin studies indicate that the cross-reaction results from similar antigenic groupings in the respective protein molecules. The number, size, and character of these common elements are as yet undetermined. Certain antisera to TMV also cross-react with normal protein preparations, but the heterologous reactions are relatively weak. The serological comparisons and the physical and chemical studies reported previously provide mutual support for the conclusion that TMV protein is derived from this particular normal protein. Antibody absorption studies indicate that some, but not all, of the antigenic groupings characteristic of the normal protein are modified in this synthesis, and that new and very effective antigenic sites specific to the virus protein are developed.

Formation of conidiophores by Helminthosporium victoriae on Vicland oats. PADDOCK, WM. C. A conidiophore fundament of *Helminthosporium victoriae*, on seedling leaves of Vicland oats (studied in cotton-blue-stained freehand sections and in whole mounts cleared with acetic acid-absolute alcohol and stained with acid fuchsin) effected egress through the epidermal cells and trichomes via one of two methods. Sixty to 90 per cent of the fundaments forcibly ruptured the outer cell wall and emerged at the leaf surface with no evident constriction at the rupture. This frequently resulted in a buckling of conidiophore fundaments within the cell in response to the pressures causing the rupture. The remainder of the fundaments formed a swelling against the outer cell wall from which a narrow, constricted hypha penetrated the wall—the reverse of an appressorium and penetration peg. Regardless of mode of egress, the conidiophores had the same final appearance, usually possessing a large bulbous basal cell on the outer epidermal cell wall surface, and on the inner surface an enlarged sub-basal cell; these gave support and rigidity to the mature conidiophore. Additional conidiophores may arise from the outer basal cell, or a second fundament may emerge through the cell wall opening made by a previous fundament.

Histological reaction of Cornellian oats to two strains of Helminthosporium victoriae. PADDOCK, WM. C. The reaction produced by two strains of *Helminthosporium victoriae* on Cornellian, a highly resistant oat, was investigated by the use of whole mounts of seedling leaves which had been dusted with spores and incubated in a moist chamber, then cleared with acetic acid-absolute alcohol and stained with acid fuchsin. Strain 1, a sporulating field strain, caused a translucent amber reaction in the epidermal cells about its appressoria, and in the adjoining epidermal cells a densely granular, deep amber cytoplasmic reaction. Primary hyphae seldom were more than 25 μ long and dis-

integrated within the epidermal cells 72 hr. after inoculation. Strain 2, a subculture of strain 1 and of greater virulence, did not cause cytoplasmic reaction or cell wall discoloration until 100 hr. after inoculation; primary and secondary hyphae 200 μ long were not unusual. These hyphae began to disintegrate 72 hr. after inoculation, but 60 hr. later some hyphae were still intact and well differentiated. The disintegration was associated with cells which had the granular, yellow-brown cytoplasmic reaction. In no case was this reaction so pronounced as that caused by strain 1.

Variation in pigment formation and other cultural characteristics of monoconidial isolates of Mycosphaerella fragariae. PALCHEFSKY, JOHN, AND C. C. ALLISON. Monoconidial isolates from typical strawberry leaf spots were grouped into two types based on cultural and substratum coloration. The substratum of the pink-red type was highly colored, whereas the gray-red type produced only slight coloration of the substratum. There were differences in other cultural characteristics within the two types. Upon successive subculturing none of the original isolates was stable. White and red-brown patches or sectors were observed in the pink-red group, and pink-red and white sectors and patches were common in cultures of the gray-red group. All monoconidial and mycelial isolates from the sectors and most from the patches were culturally similar to the patch or sector. The white and red-brown isolates were stable upon subculture. No coloration of the substratum of the white isolates was observed, but abundant substratum coloration occurred in the brown-red cultures. Monoconidial isolates from a spot on one leaflet were all gray-red, whereas the isolates from a spot on the opposite leaflet of the same leaf were all pink-red. Qualitative tests indicate that the substratum pigment is an anthocyanin. Its production is correlated with the presence of iron and zinc. The pink-red, gray-red, and white isolates caused typical strawberry leaf-spot lesions.

Scab of Chinese Holly (Ilex cornuta). PLAKIDAS, A. G. A striking, and apparently undescribed, disease of Chinese holly (*Ilex cornuta*) characterized by lesions on leaves, shoots, and berries has been observed in Louisiana in recent years. There are two distinct types of leaf lesions: (1) numerous small black spots, discrete or coalescing, on the upper surface, and (2) a large, elongate, carbonous lesion on the upper surface, usually confined to one half of the lamella and causing marked distortion of the leaf. On the shoots and berries, the lesions are brown to gray with somewhat raised margins. *Sphaceloma* sp. has been isolated from both types of leaf lesions as well as from those on shoots and berries. *Elsinoe*-like ascomata have been found in some leaf lesions, but these were not mature enough to permit description. Inoculations with pure cultures resulted in infection in a limited number of experiments. The origin of the disease has not been determined. A limited survey of American holly (*Ilex opaca*) trees, in gardens and in the wild, did not show the presence of the disease on this host.

Infection in seed embryos and seedlings of wheat and barley in relation to development of loose smut. POPP, W. The relative prevalence of loose-smut infection was determined in seed embryos, seedlings, and adult plants of varieties of wheat and barley inoculated with races of *Ustilago tritici* (Pers.) Rostr. and *U. nuda* (Jens.) Rostr., respectively. To detect the presence of loose-smut mycelium, seeds and excised portions of seedlings were macerated in a solution of potassium hydroxide. Embryos and seedling tissues prepared in this manner were boiled in a solution of cotton blue in lactophenol, mounted in glycerine-water, and examined under the microscope. Infected wheat seeds may or may not produce smutted plants depending upon the race of smut infecting them. Seedlings generally gave a more reliable index of adult-plant infection than did embryos. A progressive decline in infection occurred in one variety during plant development. A variety of wheat may be highly susceptible in the embryo stage to a race of loose smut and highly resistant during plant development. The prevalence of loose-smut infection in seed embryos or seedlings of resistant and susceptible varieties of barley was similar to that in adult plants.

Peach brown rot: Relative effectiveness of various spray applications for control in the orchard. POULOS, P. L. The following application schedules were block-tested during 1949 in Delaware in an abandoned Elberta orchard: (1) unsprayed; (2) bloom (March 23, 25, 28, 30, April 6) + pre-harvest (July 18, 23, 29, Aug. 4, 9); (3) bloom + summer (June 7, 20, July 8) + pre-harvest, and (4) pre-harvest. All blocks received cover spray applications (April 27, May 6, 16, 26). Fungicides used were: bloom—Flotation Sulfur (12–100); cover—Magnetic 70 Paste (4–100); summer—Sulforon X (6–100); pre-harvest—Sulforon X (5–100). Blossom blight infection (May 6) was 53 per cent in the unsprayed and 23 per cent in blocks receiving bloom applications. When

pre-harvest applications began (July 18), incidence of fruit rot was estimated at 5–10 per cent in unsprayed blocks. Fruit rot control data (Aug. 4, 8, 11) showed the following percentage rot for the various schedules: unsprayed, 56; bloom + pre-harvest, 8; bloom + summer + pre-harvest, 2; pre-harvest, 65 (LSD at 5 per cent = 17). These data indicate that pre-harvest applications afford no control, that summer applications in a complete schedule aid in control, and that bloom applications apparently are the most important control applications.

Studies on inhibition of virus infection by filtrates from fungus cultures. PRICE, W. C., AND B. M. GUPTA. Of 49 species of fungi grown on cornmeal-beef-dextrose broth, ten produced materials capable of reducing infectivity of Southern bean mosaic, tobacco mosaic, or tobacco necrosis virus by 85 per cent or more. The inhibitory substance of *Trichothecium roseum* inhibits infection when mixed with a virus preparation or when applied to host plant leaves either before or up to 30 min. after inoculation. Virus preparations rendered noninfectious by addition of filtrates of *T. roseum* regain activity on simple dilution, indicating either that the material enters into a reversible combination with virus, or alters the susceptibility of the host. Two lines of evidence are available to indicate that host susceptibility is altered. First, the degree of inhibition depends not only upon the virus but also upon the host plant used. Growth products of *T. roseum* which reduced infectivity of tobacco mosaic virus on *Nicotiana glutinosa* or Early Golden Cluster bean plants by 97 per cent, reduced infectivity only 4 per cent in plants of Holmes' necrotic type tobacco (*N. digluta* × *N. tabacum*). Second, the nature of the dilution curve obtained by inoculation of plants with solutions containing varying quantities of fungus growth products and a constant amount of virus is best interpreted on the assumption that the inhibitory substance alters susceptibility of the host.

Measuring efficiency of Bordeaux application. RICH, SAUL. Does more copper in the spray tank mean a proportionate increase of copper on the leaf surface? If this were true, then all concentrations of Bordeaux would be equally efficient in building up copper deposit on foliage. That this is not the case is evident from the data of Hopperstead *et al.* (1943), measuring Bordeaux residue on apple foliage. In their data the copper deposit builds up as the log of Bordeaux concentration. Our data for Bordeaux on beans and celery foliage bear this out. An efficiency index of application (E_a) may be expressed as follows:

$$E_a = \frac{\dfrac{\text{Copper deposit for test concentration}}{\text{Copper deposit for standard concentration}}}{\dfrac{\text{CuSO}_4 \text{ (lb. per 100 gal.) in test concentration}}{\text{CuSO}_4 \text{ (lb. per 100 gal.) in standard concentration}}}$$

In all data examined, efficiency of application is inversely related to concentration. Tenacity coefficients (T_c) were also obtainable from the same raw data.

$$T_c = \frac{\text{Copper deposit after weathering}}{\text{Copper deposit immediately after spraying}}$$

Tenacity improves with increasing concentration up to a certain point, after which it levels off. Multiplying E_a by T_c gave a measure of the optimum Bordeaux concentration for obtaining the largest proportionate increment of weathered deposit. This optimum was between 2–1–100 and 4–2–100 Bordeaux.

Factors influencing sporulation of Septoria nodorum in culture. RICHARDS, GERALD S. *Septoria nodorum*, the cause of glume blotch of wheat, which hitherto has not been reported to sporulate on synthetic media, has been found to fruit readily on a number of such media containing agar, provided the external environment is favorable. Galactose and glycine were the most favorable sources of carbon and nitrogen. The fungus sporulates only when temperature is maintained at approximately 20° C. Light is also necessary. Maximum sporulation resulted when cultures were continuously exposed to 100 foot-candles of illumination. More pycnidia and conidia formed when plates were inoculated by flooding with a heavy spore suspension than when minced mycelium or point inoculation was used.

Thread blight of Elaeagnus pungens. ROBERTS, DANIEL A. A thread blight, caused by the fungus to be described as *Rhizoctonia ramicola*, has been observed in Gainesville, Florida, on *Elaeagnus pungens*, *Pittosporum tobira*, *Lagerstroemia indica*, *Feijoa sellowiana*, and *Erythrina herbacea*. The fungus, unassociated with the soil as to origin, causes cortical necrosis of petioles and young twigs, and tan necrotic lesions with purple-

brown margins on leaf blades. When abscission occurs, fallen leaves frequently are matted together by mycelial wefts or held dangling by fungous threads. The fungus is characterized by its aerial habitat, short and delicate olivaceous-brown threads on the surfaces of infected petioles and twigs, and the absence of sclerotia and spores. A lavender coloration of the mycelium occurs in mature cultures on potato-dextrose agar. In culture, optimum pH for growth is near 6.3, and cardinal temperatures are 11°, 30°, and 37° C. Ingress occurs through stomata, lenticels, and parenchymatous basal cells of scales. The mycelium is intercellular. Infection depends upon an adequate source of inoculum and high daytime temperatures, accompanied by mean relative humidities of 75 per cent or more. The fungus overwinters as mycelium in leaf lesions and diseased petioles and twigs. Infection recurs annually in previously infected plantings.

Local lesion formation and virus production following simultaneous inoculation with potato viruses X and Y. ROSS, A. FRANK. Although U.S.D.A. potato seedling 41956 is considered to be immune from potato virus X, lesion formation on this host by potato virus Y is partially inhibited if the inoculum contains virus X. The number of lesions is progressively reduced as the amount of virus X is increased. If plants of *Physalis floridana* are inoculated simultaneously with virus Y and a non-lesion-forming strain of virus X, Y-lesions develop, but at a slower rate than if virus X is absent. The reaction to virus Y of *P. floridana* plants systemically infected with a masked strain of virus X is identical to that of comparable healthy plants, except that Y-lesion formation is not affected by the presence of virus X in the inoculum. The juice of *Nicotiana glutinosa*, tobacco (*N. tabacum*, var. Turkish) or potato (*Solanum tuberosum* var. Placid), infected with viruses X and Y may contain much greater amounts of virus X than do juices of comparable plants infected with virus X alone. With mild strains of virus X, the difference may be five-fold, but with severe strains it is much less or nonexistent. Virus Y concentration is unaffected by the presence of virus X.

Seasonal and cyclic variation in amount of phloem necrosis in lemon trees affected by decline. SCHNEIDER, HENRY. At varying ages after reaching a productive size in southern California, many lemon trees decline or occasionally collapse for unknown reasons. The width of functioning phloem just above the bud unions of trees in a number of orchards was measured at 3-month intervals. In normal-appearing trees of some orchards, the phloem necrosis has been extensive and prolonged enough to cause utilization of reserve starch in the xylem of finger-size roots, and a subsequent decline resulted. In trees from a warm interior valley, starch disappeared concurrently with necrosis, but trees declined subsequently. Such trees may form sufficient new phloem to make a complete or partial recovery. Trees with phloem necrosis, growing on sweet-orange stock, do not deplete their reserve starch as readily as those on grapefruit stock, but they may go into a mild decline with some starch present in the roots. In general, necrosis is most extensive in spring, but cycles of extensive unseasonal necrosis may occur any time. Although some necrosis is found in all but very vigorous lemon trees, it may not be of sufficient extent and duration to cause depletion of stored starch and its effect, if any, is obscure.

The pathological anatomy of bud-union tissues of orange trees and its value in the diagnosis of quick decline. SCHNEIDER, HENRY, J. M. WALLACE, AND J. E. DIMITMAN. In orange trees showing the nonspecific top symptoms of quick decline, radial sections disclose a series of changes in the phloem at the bud union. Below the bud union, in the older phloem which would normally be functioning, sieve tubes are necrotic and definitive callus is removed, parenchyma cells may be hypertrophied, and fat globules lysed. Just above, in the scion, seive tubes are collapsed but callus is still present, and fat globules are present in the parenchyma. Phloem produced at the union during the disease is excessive, cells are smaller than normal and usually no fibers are present. Sieve tubes below the union are necrotic, with their sieve plates callused, whereas shortly above a band of normal phloem occurs. An anatomical analysis of 61 trees suspected of having quick decline showed 46 positive, 7 possible, 3 indeterminate, and 5 negative cases. To date, bud transmission tests from these trees show that in these categories 41, 6, 0, and 0 are positive cases. Anatomical analysis is therefore of value for rapid indexing of suspected trees, particularly for quarantine purposes. Used with other symptoms it is a practical substitute for the more exacting bud transmission test which requires many months.

A Valsa associated with the decay and discoloration following the tapping of sugar maple trees. SCOTT, W. W., AND T. SPROSTON, JR. It has been known for 50 years that extensive areas of decay and discoloration often follow the tapping of sugar maple trees. Isolations made from such areas, which extend several feet above and below the original tap-hole, revealed the presence in over 90 per cent of the samples examined of a fungus

identified as a species of *Cytospora*. Cultural studies dealing with the life cycle of this organism have been made. Experimental inoculations of sugar maple quarter-sections in the laboratory resulted in the production of typical pluriloculate pycnidia bearing hyaline, allantoid conidia. The Cytospora and the Valsa stages have been found fruiting abundantly on dead and dying branches of sugar maples in the field. The Valsa stage has not yet been produced in culture. Field inoculations of young untapped sugar maple saplings resulted in typical symptoms of decay and discoloration after only 6–8 months. The fungus was reisolated from these areas. Control measures involve the design and construction of a new type of sap-spout as a means of protection.

Ceratostomella ulmi types in relation to the development of perithecia. SHAFER, THELMA, AND O. N. LIMING. Perithecia of *Ceratostomella ulmi* were found on naturally infected elm collected in New Jersey and Colorado. Perithecia, asci, and spores were smaller than those resulting from artificially paired isolates. Isolates from perithecia found in nature, after a few months in the laboratory, failed to produce perithecia. Fifty-four mass isolates were tested for their ability to produce perithecia, when alone or paired, on elm wood. Twenty-eight Type A isolates from 12 States in 384 pairings failed to produce perithecia. Nine Type B isolates from the Indianapolis, Indiana, area, paired 46 times, failed to produce perithecia. In 240 paired cultures of Type A with Type B, 217 produced perithecia. Eight isolates with Type A characteristics produced perithecia 10 times in 265 pairings with Type A isolates. Four isolates, resembling Type B, produced perithecia 10 times in 73 pairings with Type B isolates. One isolate from Connecticut and one from Ohio produced perithecia in most instances when paired with Type A and with Type B isolates. On one occasion, one of these isolates produced perithecia when not paired. Two isolates that were bisexual at first, later showed only Type A characteristics. Most type A isolates on a potato-sucrose-agar medium produced typical colonies of *C. ulmi*. Type B isolates tended to be atypical. Type B conidiospores were largely within the lower limits of the range of sizes reported for Type A spores.

Organic fungicides for control of dollar-spot disease of bent grass. SHARVELLE, E. G., AND D. S. LIKES. Dollar-spot disease (*Sclerotinia homeocarpa*) is an annual problem on bent grass greens in the middlewest. In 1949 eleven fungicides were tested for their value in rehabilitating experimntal greens heavily infected with dollar spot. Four applications of fungicides were made beginning July 6 to replicated 48-sq.-ft. plots uniformly infected prior to the first spray application. Fungicides were applied with a power sprayer at the rate of 10 gal. per 1000 sq. ft. turf at weekly intervals. A modification of the "area grade" method of rating turf fungicides was used prior to each spray application and at the end of the growing season. Six materials gave 100 per cent recovery from dollar spot four weeks after the first spray application: Crag 531 (calcium zinc copper cadminate), Cadminate (an organic cadmium), Puraturf 177 (*p*-amino phenyl cadmium dilactate), Puraturf GG (2.4 per cent mercury, 6 per cent cadmium), Puraturf (phenyl mercury triethanol ammonium lactate), and Calo-Clor (mercuric and mercurous chloride). PMAS (phenyl mercury acetate), Cadmium H.258 (an organic cadmium), and Tersan (tetramethyl thiuramdisulfide) were less effective, producing 80–90 per cent disease-free turf. Phygon (2,3 dichloro-1,4-naphthoquinone) and Spergon (tetrachloro parabenzoquinone) were ineffective for dollar spot control. Sixty per cent of the turf area in unsprayed plots was infected with dollar spot when the results were recorded.

The genera of the Peronosporaceae. SHAW, CHARLES GARDNER. A morphological character of importance in distinguishing between genera of the Peronosporaceae seems to have been neglected. The conidia of species of *Peronospora* do not possess a pore (*i.e.*, are nonporoid); correlated with this is the direct method of germination found in all species of the genus. Some species of *Peronospora* (*e.g.*, *P. destructor*) are papillate; even these, however, are nonporoid. Except for the genus *Sclerospora*, all species included in other genera of the Peronosporaceae possess poroid conidia. In many species the conidia are also papillate, but this character, which has been utilized in the past, is not constant. Poroid conidia typically germinate indirectly in contrast to the direct germination of conidia of the Peronospora type. The poroid condition of the conidia and the indirect method of germination would seem to be the more primitive condition within the family. If the usefulness of this character in distinguishing between *Peronospora* and other genera of the Peronosporaceae is accepted, the genus *Sclerospora* as now constituted needs revision. *Sclerospora* contains some species with apically poroid conidia which germinate indirectly and other species with nonporoid conidia which germinate directly. It therefore seems advisable to erect two sections within *Sclerospora*, one for those species with nonporoid conidia and one for the type species, *S. graminicola*, and the other species with poroid conidia.

A technique for measuring water loss from culms and leaves of Gramineae. SHURTLEFF, M. C. Three 7-in. portions of field-grown wheat culms or 5 seedling leaves were placed in 5 cc. of water in vials of 7 cc. capacity at the periphery of a rotating turntable. Three 300-watt Mazda bulbs suspended 9 in. over a water filter that was 7.5 in. above the turntable provided daytime illumination. Transpirational loss was determined by weighing the plants plus vials hourly, to the closest mg., for 4-hr. periods during the day and night, on an analytical balance. With practice, a weighing could be made each minute. Because water loss from a vial rarely exceeded 2.5 gm., it was unnecessary to replenish water. Convection currents were kept at a minimum, while temperature and humidity were reasonably constant for the 4-hr. test period. Three nonrusted culms lost approximately 0.6 gm. during the day period and 0.2 gm. at night. Water loss of rusted wheat culms varied directly with the amount of stem rust present. Culms with 65 per cent rust lost 200 per cent more water (1.37 gm.) during the day than did nonrusted culms (0.44 gm.), and at night 300 per cent more (0.79 gm.) than the nonrusted (0.17 gm.).

Concepts of fungus taxonomy and nomenclature. SNYDER, WILLIAM C., AND H. N. HANSEN. Biological investigations in all classes of fungi, especially in the applied fields of agricultural and industrial mycology, have accumulated overwhelming evidence that fungi are variable; yet traditional mycology, to a considerable extent, remains geared to a concept that a fungus species is static and that the basis of a species is the individual. It is proposed, in principles set forth to adjust concepts in taxonomy and in nomenclature so that they conform with present-day knowledge of variation, accomplish a reduction in the present number of species, and suit those who work with living fungi.

Electron micrography of the Wisconsin pea streak virus. STAHMANN, MARK A., D. J. HAGEDORN, AND W. C. BURGER. The high tolerance to dilution and resistance to aging *in vitro* of the Wisconsin pea streak virus suggested that it might readily be purified and studied with the electron microscope. Concentration of the virus by high and low speed centrifugation of sap extracted from severely infected Perfected Wales pea plants yielded highly infective pellets. Uranium-shadowed electron micrographs prepared from these pellets revealed a predominance of elongated, threadlike, flexuous particles. These particles were abundant in pellets prepared from plants showing severe symptoms; much less numerous in mounts from plants at early stages of infection; and completely absent in mounts from healthy pea plants. It was presumed that these elongated particles represent the Wisconsin pea streak virus. The width of the particles measured about 14 $m\mu$; however, the length was extremely variable. Threadlike particles as long as 5000 $m\mu$ and as short as 150 $m\mu$ have been observed, with no apparent predominance of a common length. These electron micrographs suggested that the Wisconsin pea streak virus exists in a much more flexible, fibrous state than viruses previously described.

Phytophthora rot of sugar-cane seed pieces. STEIB, R. J., AND S. J. P. CHILTON. A rot of sugar-cane seed pieces was found in Louisiana from which three types of *Phytophthora*-like cultures were consistently isolated. One of these has been identified as *P. erythroseptica*. The other two have not been identified. The disease was associated with poor drainage and relatively low temperatures. In inoculation experiments symptoms similar to those found in the field were obtained, and it was further demonstrated that high soil moisture was necessary for severe infection of sugar-cane stalks. The disease was widespread throughout the State.

Effect of differential felling and storage upon the quality and yield of paper pulp from birch wood. STEINMETZ, F. H., M. T. HILBORN, AND CHING-YUIN HSU. In 1941 sound paper-birch trees were felled in March, July, and September, and the July-felled wood was divided into peeled and unpeeled portions. The wood was stored in place in cord wood style. From samples taken each autumn for 4 successive years paper pulp was made. The yield, based upon dry weight, remained relatively constant until advanced stages of decay were reached. The wood felled in September remained superior for pulp over that felled in March or July. At the end of 4 years July-cut samples of peeled wood had lost 7 per cent of their original pulp value, while the loss for unpeeled wood was 21 per cent for March-cut samples, 20 per cent for July-cut samples, and 14 per cent for those cut in September. As a measure of quality of pulp, hand sheets of pulp were prepared from the original samples of 1941 trees felled in March, July, and September and compared with pulp made from peeled and unpeeled samples taken from the woodlot in February, 1949. These tests showed that peeled birch wood produced good quality pulp, while birch wood stored with bark on produced very low yield of decidedly inferior pulp after 8 years of woodlot storage.

Fungicidal synergism between urea and sulfur. STODDARD, E. M. The fungicidal activity of sulfur plus urea is greater than that of sulfur alone. Against *Sclerotinia* a mixture of sulfur and urea at the L.D. 50 level was approximately twice as effective as sulfur alone. Against apple-scab conidia on glass slides, a mixture of sulfur and urea at the L.D. 50 level was also approximately twice as effective as sulfur alone. A standard amount of sulfur plus urea gave much better control of scab in the field than sulfur alone. Improved growth and color of the sprayed foliage indicates that N is absorbed. Normally an increase in foliage N increases susceptibility to scab. The improved control in this case is evidence of fungicidal synergism between sulfur and urea. Inoculation tests indicated that leaves so sprayed were protected for a considerable time. This indicates that some tenacious fungitoxic compound may be formed on the leaf. Any unchanged urea on the leaf would be quickly absorbed or washed off and could activate the sulfur for only a short time. Inasmuch as the mixture showed increased fungitoxicity on glass slides, it is postulated that the added protection of foliage was not due to any chemotherapeutic action consequent to absorption of the urea. It is suggested from these tests that there is evidence of synergism between urea and sulfur.

A comparison of corn rootworms and Pythium graminicola as corn root pathogens. SUMMERS, THOMAS E. Two major contributors to the syndrome of corn rootrot are *Pythium graminicola* Subr. and southern corn rootworm. Comparisons were made of the relative destructiveness of *P. graminicola* and the larvae of *Diabrotica duodecimpunctata* Fab. alone and in combination. Corn plants grown in soil infested with corn rootworm alone were damaged more severely than were plants grown in soil infested with *P. graminicola* alone or in soil infested with both. There were no significant differences in number of brace roots developed, diameter of stalk or stalk height, between check plants, plants grown in soil infested with *P. graminicola*, and plants grown in soil infested with a combination of *P. graminicola* and corn rootworm. Total root weight was reduced about equally by *Pythium* alone and by a combination of *Pythium* and rootworm; the greatest reduction in root weight was by rootworm alone.

Variation of monosporous isolates and reisolates of Colletotrichum phomoides. SWANK, GEORGE, JR. The results of a study of several hundred monoconidial isolates from tomato anthracnose lesions have demonstrated that *Colletotrichum phomoides* consists of many races. These differ in frequency of sectoring, pathogenicity, abundance of sclerotia, coloration, rate of growth, and other characteristics. In some cases the cultural characteristics of reisolates from tomato fruits were different from the isolate used as inoculum. A wide range of lesion types occurs as a result of fruit inoculations with isolates that differ culturally. One fruit lesion was observed with a white sector. Monoconidial isolates from an affected green fruit produced perithecia with viable ascospores. Single-ascospore isolates produced an anthracnoselike spot of tomato fruits but no injury to apple fruits. A stock culture of *Glomerella cingulata* produced a fruit decay of both apple and tomato. Monoconidial reisolates from one tomato lesion produced perithecia and viable ascospores in culture. The perithecia, asci, and ascospores were different from those arising in the isolates from the green fruit.

Parathion therapy of meadow nematode-infected boxwoods. TARJAN, A. C. In the first of three experiments investigating the nematodical efficacy of parathion, 0.5, 1.0, 1.5, 2.0, and 2.5 gm. of the 25 per cent wettable powder in 50 cc. of water were applied as a soil drench to 5-month-old, meadow nematode-infected English boxwoods in 5-in. pots. Approximately 2 months after treatment, meadow nematode counts for each plant were obtained by a modification of the Baermann technique. The 2.5-gm. treatment appeared extremely effective in reducing nematode populations. In the second experiment, 2.5, 4.0, 5.5, 7.0, and 8.5 gm. of the powder were applied as before. Treatments of 4 gm. and higher of parathion per plant proved phytotoxic in some cases; the 8.5-gm. treatment killed all plants. Root-nematode counts indicated that the reduction of meadow nematodes in the 2.5-gm. treatments was highly significant in comparison to the controls. In the final experiment, 1.0, 1.5, and 2.0 lb. of the chemical were applied within a 2.5-ft. radius around the trunks of 14-year-old nursery-grown boxwoods. All plants received 3 gal. of water. Counts of meadow nematodes in roots of plants receiving parathion treatments were lower at the one per cent level of significance than comparable counts from the controls.

Spray timing in the control of apple fruit spot, sooty blotch and flyspeck. TAYLOR, CARLTON F. Applications of Bordeaux mixture (1–2–100) and a Fermate (1–100) were made on Rome Beauty apples using five periods at 18-day intervals in 1949. The spray schedule was designed to determine the spray date most critical in control of sooty blotch. Fermate (ferric dimethyl dithiocarbamate) applications were duplicated on Golden De-

licious. In 1948, the experiment covered all combinations of seven periods at 14-day intervals using ½–2–100 Bordeaux mixture on Rome Beauty. Fermate was tested only in combinations of two applications at 28-day intervals. In 1949, all applications of Bordeaux mixture from May 27 (first date) through July 2 gave good control of fruit spot; later applications had little effect. Fermate timing was similar but the control was slightly less effective. With flyspeck the most effective date was July 19 with both materials. Sooty blotch control with Bordeaux mixture was best in mid-July in both 1948 and 1949. With Fermate, control was poorer, with the most effective date in late May and decreasing control at later dates. These data suggest a difference between low concentrations of Bordeaux mixture and Fermate in the mode of action in control of sooty blotch.

Destruction of crown-gall bacteria in periwinkle by high temperature with high humidity. THEIS, T. N., O. N. ALLEN, AND A. J. RIKER. Failure consistently to kill crown-gall bacteria, *Agrobacterium tumefaciens* (Smith and Town.) Conn., in periwinkle (*Vinca rosea* L.) by 47° C. for 3 days suggested that not all the critical factors were understood. *In vitro*, the bacteria did not survive the treatment. *In vivo*, viable bacteria were recovered after 5 days at 48.5° C. with relative humidity of 40 to 50 per cent. The internal plant temperatures approximated those of the heat chamber. The incubation periods were varied without changing results. Inoculations with a few washed cells gave similar results. The persisting bacteria were inside isolated pockets. Complete elimination of the bacteria was obtained with relative humidity above 65 per cent during heat treatment. Under these conditions, however, the galls became heavily contaminated with fungi, chiefly a *Penicillium*, which produced an antibiotic against crown-gall bacteria. Aseptic technique freed inoculations from extraneous microorganisms. Stems were enclosed in glass cylinders and surface-sterilized, inoculated, and heat-treated at 46.5° C. for 3 days at 85 per cent relative humidity. Under these conditions, large fungus-free and bacteria-free galls developed. Both bacteria and plants withstood high temperatures best with low humidity.

Seed treatment of safflower and varietal susceptibility to Alternaria blight. THOMAS, C. A. Seed treatment studies were made in the greenhouse and field at Beltsville, Maryland, on the control of seed rot and damping-off of safflower caused by *Alternaria carthami*. Ceresan M (ethyl mercury p-toluene sulfonanilide), New Improved Ceresan (ethyl mercury phosphate), Arasan (tetramethyl thiuramdisulfide), Semesan (hydroxymercuriehlorophenol), Spergon (tetrachloro-para-benzoquinone), Phygon (2,3-dichloro-1,4-naphthoquinone), and Seedox (trichlorophenyl acetate) were applied as dusts at the rate of 2.5 oz. per bu. to naturally infested seed. Ceresan M and New Improved Ceresan gave complete control and resulted in a 100 per cent increase in stand. No other material tested was effective. Safflower plantings made with disease-free seed in isolated plots in Maryland and Nebraska developed severe leaf spot. Fifty-four varieties and selections were tested in the greenhouse for susceptibility to blight by artificial inoculation of seedlings. Nebraska variety N 9 and six selections were highly resistant. Under field conditions in western Nebraska, however, several varieties susceptible in the greenhouse test appeared resistant. In a fungicide test at Beltsville, Parzate (zinc ethylene bisdithiocarbamate) gave much better control of leaf spot than either Fermate (ferric dimethyldithiocarbamate) or Copper A (copper oxychloride) under conditions of heavy infection.

Red node, a virus disease of bean. THOMAS, H. REX, AND W. J. ZAUMEYER. Red node, a virus disease of beans, occurs erratically in the bean-growing sections of the Mountain States. In the field it causes reddish discoloration of the nodes and pulvini of the stems and leaf petioles, and concentric ringed patterns on the pods. The virus is infectious by mechanical transmission with carborundum to tobacco, cucumber, jimsonweed, guar, pea, soybean, crimson clover, white sweet clover, broadbean, vetch, and cowpea. It was inactivated *in vitro* between 56° and 58° C. and tolerated a dilution of 1 to 500 but not 1 to 1000. Rapid partial inactivation of the virus occurred after storage for 20 min. at 22° C. *in vitro*. The virus was infectious *in vitro* after 24 hr. but not after 48; it resisted drying in leaf tissues for 30 but not for 90 days. Limited evidence indicates that the virus is not seed-transmitted. No resistance was found among 25 bean varieties artificially inoculated. Based on similarity of virus properties, on symptoms in beans, jimsonweed, and Turkish tobacco as well as other hosts, and on limited cross-protection tests, it is concluded that the red node virus is related to the tobacco streak virus.

Manganese toxicity in peaches. THORNBERRY, H. H. A new disease of the inner bark of trunks and larger branches of peach trees was reported in 1946 by growers in southern Illinois. It is tentatively designated as "internal bark necrosis." The early lesions of pin-point to 2 mm. size are localized internally without any observable change on the surface. Isolated lesions either enlarge as separate spots to about 10 mm. in diameter, or converge into necrotic areas which tend to advance until the trunk or branch is girdled. At later stages of disease there is surface darkening and subsequent splitting of the outer bark, along with the production of gum. Microorganisms were not found in the necrotic tissues. Excessive manganese appeared to be a more plausible cause of the disease than a virus or winter injury. Manganous sulfate injected into trees produced internal bark necrosis similar to that found in affected trees. Amounts of manganous sulfate sufficient to cause injury were 1 gm. per disease-free tree growing in the affected area, and 2 gm. per tree growing in a nonaffected area. From these results it is concluded that excessive manganese is involved in the internal bark necrosis.

Chemical treatment of tobacco plant bed soils for weed and disease control. TODD, F. A., G. B. LUCAS, AND E. L. MOORE. Weed and disease control is essential to successful production of tobacco plants on old plant bed soils. Soil fumigation with methyl bromide gas (1 lb. per 10 sq. yd.) was effective against weeds and black shank (*Phytophthora nicotianae*) and reduced root knot (*Heterodera marioni*). When applied at double this rate, root knot was effectively controlled. Methyl bromide solution (15 per cent by volume in xylene) emulsified in water and applied as a drench (255 ml. per gal. per sq. yd.) was effective against weeds and black shank but not root knot. However, when chloropicrin was added to this emulsion (85 ml. per gal.) root knot was controlled. Chloropicrin emulsion (38 ml. per gal. of water per sq. yd.), when applied as a drench and followed by immediate covering of the soil with sisalcraft paper, controlled black shank but not weeds. Allyl alcohol (6 and 8 qt. per 100 gal. water), applied at the rate of 1 gal. per sq. yd., effectively controlled weeds and, when applied in the spring 2 weeks before seeding, reduced root knot. Fall treatments (Cyanamid 1 lb. + sodium azide ¼ lb. per sq. yd. and sodium nitrite-nitrate 1 lb. per sq. yd.) controlled nematodes and weeds.

The infectious nature of crazy top of corn. ULLSTRUP, ARNOLD J. During the past growing season crazy top of corn appeared to be more abundant in Indiana than in the past several seasons. In all cases the disease occurred in areas in fields that had been water-logged after planting. Seventy to 80 per cent of the plants in 5 to 7 acres of a 20-acre field were infected. Histological sections of affected plants showed a sparsely developed coenocytic mycelium in all tissues. Characteristic mycelium was found in the embryos of the kernels of those few plants that produced ears. In leaves and leaf sheaths, oospores were found that were typical of the genus *Sclerospora*. Sporangia were not found on plants in the field or on those transplanted and held in a saturated atmosphere at 15° to 18° C. Based on the symptoms of downy mildew of corn as reported from Italy, the size and character of oospores, and the failure of the pathogen to produce an asexual stage, it is tentatively concluded that crazy top of corn in the United States is caused by *Sclerospora macrospora*.

Some factors affecting appressorium formation and penetrability of Colletotrichum phomoides. VAN BURGH, PATRICIA. Formalized gelatin membranes of 5 degrees of hardness were used to investigate certain factors affecting the penetrability of *Colletotrichum phomoides*. When spores were sown in water on membranes placed on water agar, penetration of the softest type occurred first, after 30 hr. No penetration of the hardest had occurred after 6 days. When potato-dextrose broth or tomato-fruit extract were included in either spore drop or underlying agar, penetration of the four softest types had occurred after 70 hr., having begun with the softest after about 20 hr. Penetration of the fifth occurred after 110 hr. With respect to appressorium formation, the contact factor is a variable one, and its effect may be modified by that of nutrient materials. No appressoria were formed on the softest membrane which was the first to be penetrated, and the number formed increased with increasing hardness of the remaining membranes. No typical appressoria were formed on any of the membranes when nutrient materials were present. Significantly more appressoria formed on the harder of two differential substrates of paraffin-petrolatum when the spores were applied in distilled water. When potato-dextrose broth was used, the number of appressoria formed on both was greatly reduced.

Cultivation of wild forms of Psalliota bispora Schäff. and Möll. WAHL, I. Two morphological types have been distinguished among the wild two-spored mushrooms of

the genus *Agaricus* collected at St. Paul, Minnesota. One type has a cream-brown scaly cap, and its stem is usually equal to or shorter than the diameter of the cap. The other type has a creamy-white, fibrillous cap and a long, slender stem. Since both types are two-spored and possess marginal cystidia on the gills, they show close relationship to *Psalliota bispora* Schäff. and Möll., and should not be regarded as the common field mushroom, *Agaricus campestris* Fr. Tissue cultures and multisporous cultures of the brown scaly type grow vigorously on potato-dextrose agar and also produce a luxuriant grain spawn. They are very prolific in horse manure fermented at high temperatures. Tissue cultures and multisporous cultures derived from the white-cream type were grown on potato-dextrose agar plus uranium nitrate (0.5 gm./l.) for several generations and produced fluffy mycelium, subcultures of which grew abundantly on potato-dextrose agar and produced sporophores in horse manure approximately 6 weeks after spawning. These new types may have commercial value after further selection.

Nature of variations induced by uranium nitrate in the cultivated mushroom. WAHL, I. Monosporous colonies of the cultivated mushroom, brown variety, on potato-dextrose agar containing 0.1–1.0 gm. uranium nitrate per liter have yielded two variants distinctly different from the parental type: a white, fluffy, fast-growing culture and a pink, flat, zonated colony. Comparative studies on 400 lines derived from hyphal tips and hyphal segments indicate that variants did not result from chance assortment of heterocaryonts; rather, they appear to be true mutants. This conclusion is supported by the fact that the variant characters usually remained unchanged in passage through the sporophore stage. The fluffy mutant was considerably more tolerant to high temperatures (30°–32.5° C.) than the parental line, whereas growth of the pink, zonated mutant was strikingly inhibited. Carbohydrate requirement of the fluffy mutant was similar to that of the original line, and both of the mutants developed in the same range of pH as the parental line. The pink, zonated mutant is markedly deficient in some growth-promoting substances present in extracts prepared from sporophores of the cultivated mushroom.

The mode of seed infection by the cabbage black-rot organism. WALKER, J. C. The association of *Xanthomonas campestris* (Pam.) Dows. with cabbage seed has long been known, but the progress of the organism in the seed plant has not been worked out completely. After infection of young plants or of those approaching the head stage, the pathogen may proceed with no external sign of disease for a considerable period. The greatest amount of seed infection occurs in very mildly affected seed plants in which external symptoms appear about the time the seed is in the dough stage. Systemic invasion then occurs by way of the xylem of peduncle, silique, and funiculus to the seed coat.

Relative importance of phenols and volatile sulfides in disease resistance in the onion. WALKER, J. C., J. H. OWEN, AND M. A. STAHMANN. Pungent and nonpungent varieties of onion in the white, red, and yellow bulb-color classes were grown in randomized blocks and inoculated with the neck-rot organism (*Botrytis allii* Munn) and the smudge organism (*Colletotrichum circinans* (Berk.) Vogl.). By means of an index for each disease the six groups of bulbs were compared. Phenols in the dry outer scales of colored bulbs and the toxic volatile components in the fleshy scales of all varieties both influenced the degree of disease resistances. The phenols were the primary basis of resistance to smudge and the volatile toxic materials were of secondary importance, while with neck rot the reverse was true.

A rare fungus disease of cereal seed. WALLACE, H. A. H. A rare fungus, *Podosporiella verticillata*, was isolated from 11 samples of seed of common and durum wheat grown in 1946 and 1947. The fungus resembles *Helminthosporium cyclops* and the imperfect stage of *Pleosphaeria semeniperda*. It is new to Canada and was found only on seed samples originating in the area extending from Assiniboia, Saskatchewan, to Edmonton, Alberta. In all instances only a trace of infection occurred. Naturally infected seed did not germinate. When infected kernels were incubated, black stalks (synnemata) up to 2 cm. in length were produced. Conidiophores may develop over the entire surface of a stalk. Each conidiophore bears at its tip one or more spores resembling those of *Helminthosporium*. When mature seeds of cereals were artificially inoculated, germination was unimpaired and healthy plants were produced. Inoculation of developing kernels in heads of wheat and panicles of oats resulted in apparently healthy seeds, but when these were placed on moist filter paper they either failed to germinate or produced stunted seedlings. Experiments indicate that this injury is caused by a toxin produced by the fungus.

New viruses naturally infectious to cowpea. WARID, W. A., AND A. G. PLAKIDAS. A large number of collections of cowpea plants with mosaic or mosaiclike symptoms was

made in the field in Oklahoma and Louisiana. By the use of 31 varieties of cowpea (*Vigna sinensis*) and 10 other species, *viz., Capsicum frutescens, Cucumis sativus, Glycine max, Medicago sativa, Nicotiana glutinosa, N. tabacum, Phaseolus lunatus* f. *macrocarpus, Phaseolus vulgaris, Pisum sativum,* and *Vicia faba* as differential hosts, and by a study of physical properties, three new viruses have been determined as occurring naturally on cowpea. These are tentatively designated as *Vigna virus 1, Vigna virus 2,* and *Vigna virus 3.* A strain of the last-named virus has also been determined. This is designated as *Vigna virus 3a.* In addition to these three viruses evidence was obtained indicating the presence of two virus complexes naturally in cowpea. The reaction of the 31 varieties of cowpea toward these viruses varied from immunity through local infection to systemic infection, but in no case was the same variety immune from all the viruses. *Vigna virus 1* was found transmissible through bean seed.

Germination of sclerotia of Sclerotium rolfsii after storage at various relative humidity levels. WATKINS, G. M. Samples of sclerotia from pure cultures of *Sclerotium rolfsii* were stored for periods of 2 to 25 days in jars in which the relative humidity was controlled at various levels from 0 to 100 per cent. Determinations of percentage and vigor of germination of sclerotia from the different treatment lots showed that most vigorous and abundant germination (91 to 98 per cent) occurred with those stored at RH levels from 25 to 35 per cent for 4 to 6 days. Notably less vigorous and less abundant germination (14 to 49 per cent) was observed after storage for similar periods at 65 to 100 per cent RH. Germination was low (3 to 26 per cent) with sclerotia subjected to very dry air (0 to 15 per cent RH). Storage of sclerotia for a few days at 25 to 35 per cent RH appears to improve the effectiveness of these bodies in inoculation experiments.

Inheritance of a necrotic lesion reaction to a mild strain of the tobacco mosaic virus. WEBER, PAUL V. V. *Nicotiana sylvestris, N. longiflora,* and a number of varieties of *N. tabacum,* which yield no necrotic lesions when inoculated with a severe strain of *Tobacco virus 1,* produce typical necrotic local lesions with the mild green mosaic strain used in this study. Three such types, namely: *N. sylvestris,* Clayton's TL106 (*N. longiflora* parentage), and *N. tabacum* var. Daruma crossed with the non-necrotic reacting variety *N. tabacum* var. Havana-Seed, yielded necrotic lesions of an intermediate or incompletely dominant type in the F_1. The F_2 ratios (1 necrotic: 2 intermediate: 1 non-necrotic) and the F_3 data from the TL106 and Daruma crosses supported this conclusion. On the other hand, F_1 and backcross data indicate that the necrotic reaction of *N. longiflora* is a completely dominant Mendelian character in a cross with a non-necrotic reacting variety of *N. alata.* When Holmes' Samsun (*NN*) was crossed with Daruma, the results indicated that the necrotic reaction of Daruma was the recessive rather than the dominant allele. Thus it is proposed that a series of multiple alleles composed at least of the gene pairs *NN*, $n^s n^s$, and *nn* exists, where "s" represents the "sylvestris type" of reaction to the mild strain.

Genetic factors controlling perithecium formation in Glomerella. WHEELER, H. E., J. W. MCGAHEN, AND S. J. P. CHILTON. Wild-type cultures of a *Glomerella* isolated from *Ipomoea* produce self-fertile perithecia in large glomerate masses. Factor pairs at four different loci have been identified which affect the formation and development of perithecia in wild-type cultures. These have been designated *Aa, Bb, Nn,* and *Ss* with the capital letters representing wild-type genes and the small letters representing their respective mutant alleles. Of the four mutant genes, only *a* completely inhibits the production of perithecia and perithecial fundaments. The mutant gene *b* determines a scattered arrangement of perithecia which are mostly self-sterile. The mutant gene *n* results in the formation of small protoperithecia and completely inhibits the development of asci and ascospores. Recombination of *A* and *N* in crosses of two nonascigerous cultures, *i.e.*, $ABn \times abN$ or $Abn \times aBN$, results in the occurrence of ascigerous cultures in the progenies. Effects similar to those produced by *n* occur when the mutant gene *s* is present. This mutant gene is highly unstable and reverts with great frequency to its wild-type allele *S*. Thus, reverse mutation at the *s* locus in *ABs* and *Abs* nonascigerous cultures results in ascigerous cultures *ABS* and *AbS*.

The proteins of green leaves. IV. Chemical and physical evidence that tobacco mosaic virus is derived from a main nucleoprotein component in tobacco leaf cytoplasm. WILDMAN, S. G., AND JAMES BONNER. The extracted protoplasm of parenchyma cells in healthy and TMV-infected Turkish tobacco leaves was fractionated into particulate matter (mainly chloroplast fragments) and soluble cytoplasmic proteins. No significant difference, resulting from virus multiplication, was detected in the quantity of either fraction. Electrophoretic analysis by the Tiselius method revealed that 40 per cent of the

total soluble cytoplasmic proteins was changed after infection to the new protein characteristic of TMV (tobacco mosaic virus). The evidence indicates that only the single, major nucleoprotein component constituting about 80 per cent of the soluble cytoplasmic proteins normally present in leaves decreases in amount as virus protein is synthesized. Virus protein, detectable electrophoretically 3 days after infection, increases steadily to a static level at about the 12th day, when it has replaced about 40 per cent of the main nucleoprotein. Thus, virus protein appears to reach a maximum before visible symptoms of the disease appear. Preliminary amino acid and nucleic acid analyses show little detectable difference in the chemical composition of virus and normal protein. The hypothesis is advanced that virus protein synthesis represents, not a complete breakdown of the normal protein followed by a new and constitutionally different synthesis, but some kind of more direct aggregation of the normal nucleoprotein.

Ethylene, a metabolic product of diseased or injured plants. WILLIAMSON, C. E. Investigation of the cause of rapid yellow coloration and early abscission of blackspot-infected rose leaves showed that such leaves produced more ethylene than healthy leaves. Ethylene production by infected leaves was greatest while the leaf was green, decreased as the leaf became yellow, and ceased when the leaf died and became brown. Ethylene was not produced by the fungus *Diplocarpon rosea* in culture. Cherry leaves with shot hole produced large amounts of ethylene. Rapid yellow coloration and early abscission of infected leaves seem to be correlated with production of large quantities of ethylene by rose leaves affected with black spot and cherry leaves affected with shot hole. Rose affected by other diseases produced ethylene but in smaller amounts than black-spot leaves. In general, diseased plant foliage produces greater amounts of ethylene than do comparable healthy tissues; but the amount depends upon the pathogen involved. Healthy tissues produce small quantities of ethylene, the amount depending to some extent upon the species of plant. Rose leaves shredded with a knife or heavily infested with red spider mite produced considerably more ethylene than did healthy rose leaves.

The protective and eradicative actions of lime sulfur and Puratized in controlling a fungus leaf-spot disease of olive. WILSON, E. E. Lime-sulfur, 3–100, and Puratized N5E (phenyl mercury triethanol ammonium lactate), 3/16–100, applied as sprays to olive leaves infected by *Cycloconium oleaginum*, destroyed many of the conidia being produced thereon and repressed further conidial development for at least a month. When applied in the autumn prior to the first leaf infection of the season, one-treatment programs of lime sulfur, Puratized, and Bordeaux (10–10–100) gave practically equal control of the disease. When infection preceded the treatments, however, and, as a consequence, new lesions with abundant conidia had developed by early February in treated trees, the November 25 applications of the three materials differed widely in preventing further infection during the spring months. The amounts of disease developing in two periods (March to May, May to June) were reduced, respectively, 95 and 87 per cent by Bordeaux, 88 and 40 per cent by Puratized, and 63 and 22 per cent by lime sulfur. Single treatments of lime sulfur given November 11, November 25, and December 16 reduced the number of new lesions devloping between March and May 43, 63, and 88 per cent respectively, whereas the corresponding percentages for Puratized were 88, 87, and 94.

Low-gallonage spray applications for the control of vegetable diseases. WILSON, J. D. Twelve fungicides were applied to tomatoes with water used at the rate of 40, 80, and 160 gal. per acre. Control of early blight on the foliage was similar for the different rates. A score taken in mid-September showed an average defoliation on the check plots of 55 per cent, whereas it was 27, 26, and 23 per cent, respectively, for the 40-, 80-, and 160-gal. applications. Anthracnose fruit rot control was somewhat, but not outstandingly, better with the 160- than with the 40-gal. application. Similar results were obtained in the control of early blight when several different materials were applied to potatoes. Some fungicides were better adapted (gave better control of early blight) to low-gallonage applications than others. The zinc carbamates in these preliminary trials seemed to give somewhat better results than the other fungicides tested. Some gave some foliage injury when used with only 40 gal. water. To check this injury factor further, six different vegetables were sprayed at 160 and 40 gal. per acre with Zerlate and with Tribasic used in combination with DDT and Parathion. The fixed copper was slightly injurious to beans when applied with 40 gal. water but neither it nor Zerlate showed any phytotoxicity when applied at this rate to cucumber, pepper, eggplant, tomato, and potato.

Yellow stipple, a new virus disease of bean. ZAUMEYER, W. J., AND H. REX THOMAS. A new virus of bean that occurred in Stringless Black Valentine was isolated from pods

collected in southern Illinois in 1948. This virus produces a mild mottling of the leaves. On most dry bean varieties, in addition to the mottling, there are formed small yellow chlorotic spots which may coalesce and form irregular patches as large as 10 mm. in diameter. Veinal and stem necroses are frequently observed. Of 36 bean varieties inoculated, none was resistant. Six varieties of peas, 9 of soybeans, and 5 of cowpeas that were inoculated were infected either locally or systemically. Three other species of Leguminosae, guar, tepary bean, and scarlet runner bean, were the only other suscepts found among 31 species representing 20 genera in 7 families. The virus was inactivated between 72° and 75° C. when heated for 10 min. It was infectious at a dilution of 1 to 50,000, but not at 1 to 75,000. It resisted aging for 5 days *in vitro* at 18°. In dry tissue stored at room temperature it was still active after 80 days. The disease caused by this virus is designated yellow stipple.

Cultural variation of monoconidial isolates of Colletotrichum trifolii. ZERKEL, ROBERT S. Two mass cultures, B and C, of *Colletotrichum trifolii* were obtained from alfalfa during the summer of 1948. During the summer of 1949 alfalfa was inoculated with culture B and alfalfa and red clover with culture C. The fungus was reisolated from the stems, and monoconidial isolates were obtained from these cultures and from the original cultures B and C. Three weeks after transfer to potato-dextrose agar, three monoconidial isolates of culture B had black subsurface mycelium, small abundant acervuli, and featherlike masses of dark marginal hyphae. The fourth isolate had black subsurface mycelium, a few large acervuli, and an abundance of gray and white aerial mycelium on the central two-thirds of the colony. The three monoconidial isolates obtained from the reisolation from alfalfa, inoculated with B, were similar to the isolate with aerial mycelium. The four monoconidial isolates from culture C and the four from alfalfa, inoculated with C, were similar to the three monoconidial isolates from culture B except for the production of large acervuli or groups of acervuli and a more definite colony margin. The five monoconidial isolates obtained from the reisolation from red clover were similar to the monoconidial isolate of culture B that had aerial mycelium.

LEAF DISEASES OF PERUVIAN BARBASCO, LONCHOCARPUS UTILIS[1]

BOWEN S. CRANDALL[2]

(Accepted for publication June 25, 1949)

Barbasco or cube, a native rotenone-producing leguminose plant of Peru and other South American countries, has been grown by the Indians for centuries for use as a fish poison. In Peru a single rotenone-yielding species, *Lonchocarpus utilis*[3] A. C. Smith, is called by the local name barbasco. Another species, *L. silvestris*[3] A. C. Smith, is found in Peru but is not valued highly and is called sacha (forest) barbasco to distinguish it from the true barbasco. In the Guianas and some parts of Brazil the rotenone-producing species is *Lonchocarpus urucu* Killip and Smith.

In comparatively recent years the increasing use of rotenone as an insecticide has resulted in the cultivation of barbasco on an extensive scale so that it is now one of the important export crops of Peru. Until recently the flowers of Peruvian barbasco had never been observed by scientists (1) and seed had never been known to be produced. The plantings made by the Indians for fish poison and the present commercial plantings are all propagated from cuttings. This concentration of large quantities of more or less clonal material could be expected to bring into prominence indigenous diseases that had gone unnoticed on the plant in its wild or semi-wild state.

The three leaf diseases here reported are apparently all native. All increase and decrease in intensity of disease is dependent upon varying factors such as weather, soil condition, and proximity of old barbasco plantings. All are true diseases in that they attack young, developing, functional leaves, causing considerable loss of leaf surface, and from time to time retarding development of the plant.

DICHEIRINIA LEAF RUST

Cause

The leaf rust of barbasco is caused by *Dicheirinia archeri* Cummins (4). This fungus was first collected in Surinam in the Guianas on *Lonchocarpus nicou* (Aubl.) D. C. and, prior to the observations here reported, had also been collected in Peru (5). This rust is autoecious and spreads from plant to plant by means of teliospores.

[1] A contribution from the Estación Central de Colonización en Tingo María, Peru, a technical agricultural service organization for the Orient of Peru, operated jointly by the Dirección de Colonización y Asuntos Orientales, Ministry of Agriculture of Peru, and by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. This study was made possible by funds provided through the Interdepartmental Committee on Scientific and Cultural Cooperation and funds from the Peruvian Government.

[2] Senior Pathologist, Office of Foreign Agricultural Relations, U. S. Department of Agriculture; and formerly Technical Chief, Department of Plant Pathology and Entomology, Estación Central de Colonización en Tingo María.

[3] Considered varieties of *Lonchocarpus nicou* (Aubl.) D. C. by Hermann (6).

Symptoms and Pathological Anatomy

Initial infection regularly occurs on the young, tender leaves before the heavy cutinized surface develops. Attack before full size is reached usually results in a distorted, malformed leaf or leaflets. More regularly

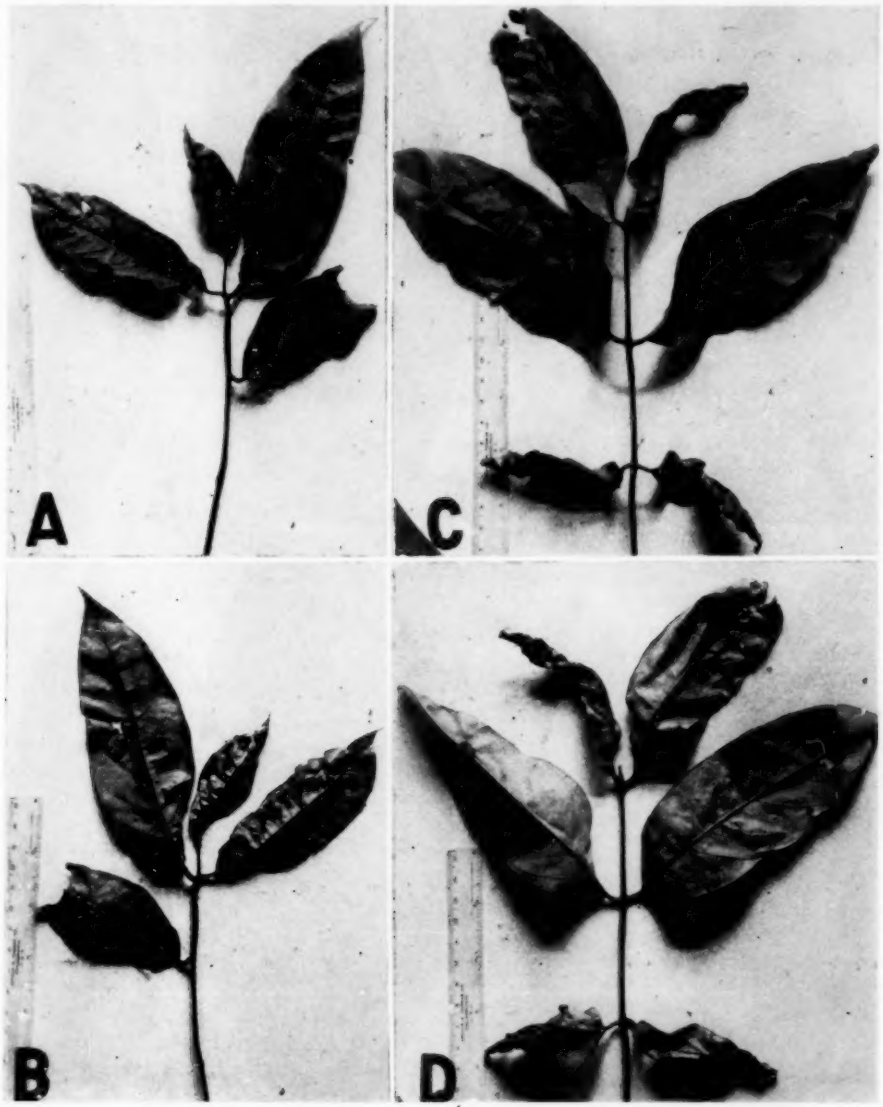

FIG. 1. A, rust infection on petiole, upper surface. B, same, lower surface. C, Cephalosporium infection, upper surface. D, same, lower surface.

infections occur, or at least pass beyond the incipient stage, about the time the leaf and leaflets are reaching full size. The infection court may be on or between the veins or midrib of the leaflets or on the midrib (Fig. 1, A

and B), with the rust showing some affinity for the veins or midrib. Interveinal infections first manifest themselves as slightly chlorotic areas soon becoming depressed on the top of the leaf but sharply raised on the lower

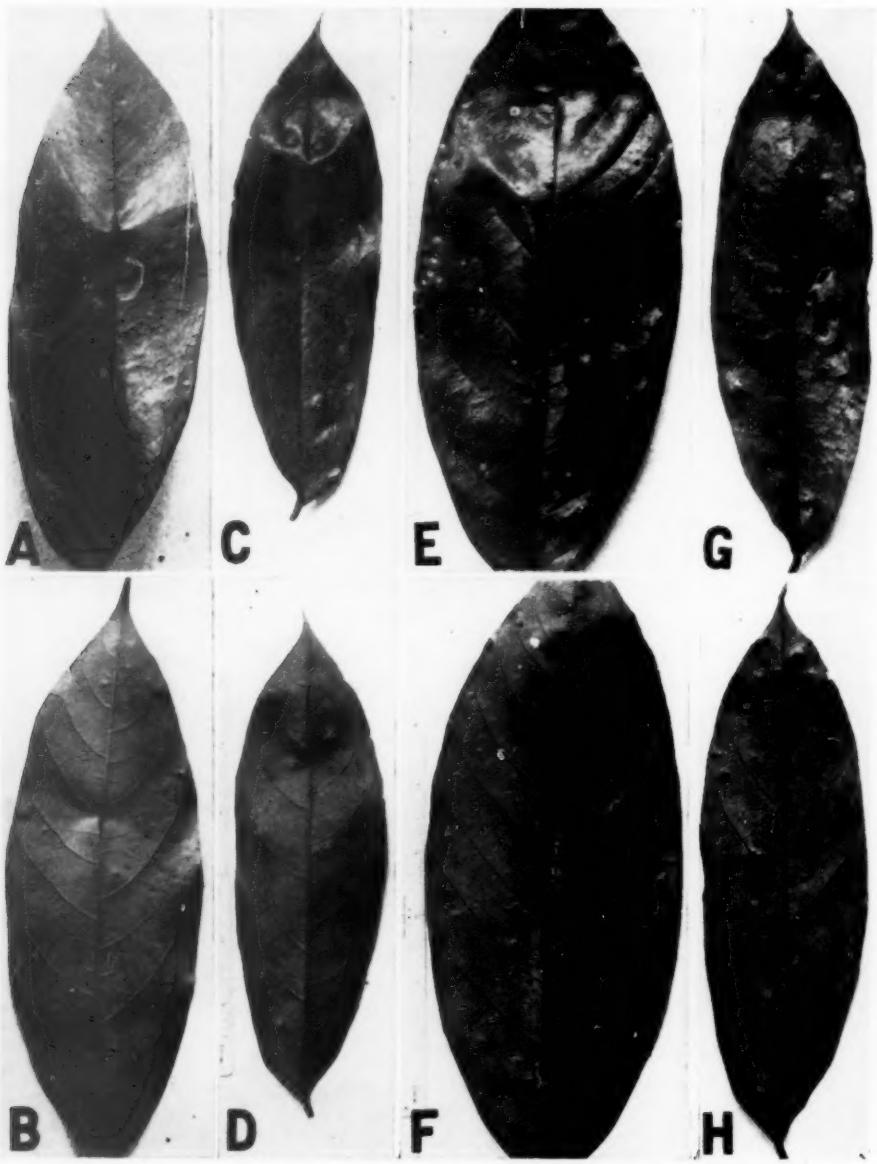

FIG. 2. *Dicheirinia archeri*. A, upper surface, and B, lower surface, early blister stage; C and D, later blister stage; E and F, prepustule stage; G and H, fruiting pustules.

surface (Fig. 2, A, B, C, and D). Infections on the veins or midrib at this stage are usually less clearly defined but tend to distort the surrounding

leaf surface. As the infection develops and the pustule starts to form, a hard, green wart, more or less ½ mm. high and slightly depressed in the center, appears (Fig. 2, E and F). The surface eventually turns brown and at the time of maturity often reaches a height of 1 mm. The upper surface of the leaf over a pustule is usually nothing more than a small, slightly depressed, necrotic area, roughly circular in shape. As the infection develops, a necrotic area surrounding the spot may develop but is usually absent except in the case of infections on the veins, when the leaf surface beyond the infection sometimes dies. About the time the leaf is hardened and mature the pustule starts to produce teliospores (Fig. 2, G and H). An occasional pustule develops on the upper side of the leaves but usually pustules develop on the under side. All vein infections are on the under side. Interveinal spots seldom exceed 2 to 3 mm. in diameter, but vein and especially midrib infections often elongate to 2 or 3 cm., eventually presenting a corky, serrated appearance (Fig. 1, A and B), and become covered with small fruiting pustules. Necrosis in infected tissue usually reaches only the phloem tissue and the water-conducting xylem remains functional.

Inoculations on older, mature leaves are unsuccessful but young, semimature leaves can be readily inoculated with spores atomized on the leaves. Typical infections can be recognized in about 10 days. Actual spore production only occurs after the leaf is fully mature, with a hard cutinized surface. Typically the period from the time a leaf opens until it reaches full maturity is some 30 to 45 days.

Hosts and Geographical Range

As previously mentioned, the original host from which *Dicheirinia archeri* was described was *Lonchocarpus nicou* in Surinam. Introductions of *L. urucu* at the Tingo María Station are more susceptible to rust than the local *L. utilis*. The rust has been reported by Garcia Rada and Stevenson (5) at San Lorenzo, Peru, and has been collected by the writer on *L. utilis* from the region of Iquitos, from Pucallpa on the upper Ucayali River, and from barbascals generally along the Ocean to Amazon Highway from Tingo María on the Huallaga River to Pucallpa. Sacha barbasco has been found heavily infected in the region of Iquitos and at this location *L. utilis* cuttings that were being rooted in the shade of heavily infected sacha barbasco were being infected before removal to the field plantings.

PELLICULARIA TARGET SPOT

Cause

Target spot is caused by a species of *Pellicularia* characterized by spores measuring 15.3–20.4 $\mu \times 5.1$–6.8 μ. Under the present concepts of the genus this species is referable to *P. filamentosa* (Pat.) Rogers (10).

Symptoms and Pathological Anatomy

Initial infection regularly occurs on young, tender leaves about, or slightly before they reach, full size. The disease first is observed as a wet, slightly chlorotic pinpoint spot, soon becoming a small circular necrotic spot (Fig. 3, A) about 1 mm. in diameter surrounded by a halo of chlorotic tissue of about the same width. Growth of the spot continues in a series of concentric circles, up to about ten, forming more or less perfect target spots (Fig. 3, B and C), usually averaging about 1 cm. in diameter. Until development of the spot is completed, surface hyphae of the Rhizoctonia stage are regularly found 1 mm. or more ahead of internal parasitized tissue. An actively enlarging spot is regularly surrounded by the chlorotic circle of recently parasitized tissue of 1 mm. in width. This apparently,

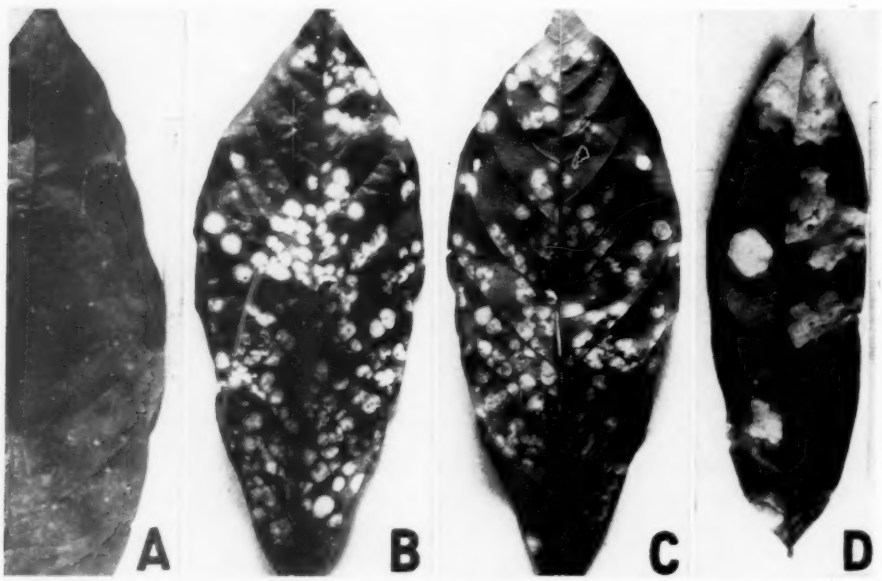

FIG. 3. *Pellicularia filamentosa*. A, incipient infections; B and C, upper and lower leaf surfaces showing 1-week-old target spots just before fruiting age; D, mature spots of fruiting age.

under normal night and day temperature and humidity, represents a 24-hr. cycle of growth. In culture the Rhizoctonia stage of all the local isolates of diseases caused by *Pellicularia filamentosa* tend to form zones. This tendency is not present to any appreciable extent in isolates of *Rhizoctonia solani* Kuehn. Furthermore, the Rhizoctonia stage of *P. filamentosa* is much slower growing in culture, 5–10 mm. per 24 hr., as compared with 20 mm. for *R. solani*. After the leaf matures and hardens, a well-defined red-brown border develops (Fig. 3, D) at the circumference of the now static spot. At about this stage or earlier, the spot develops a silvery, powdery appearance on the lower surface of the leaf, and during periods of wet weather or high humidity abundant spore production occurs. Occasional

spurts of growth occur on older spots on older leaves, giving them an irregular appearance in the outer zone. Where many spots occur on the same leaf they often coalesce (Fig. 3, D). Single spots seldom exceed 2 cm. in diameter but multiple infections often kill 50 per cent and more of the leaf surface. In badly infected plantings growth apparently is retarded when more than half the leaf surface of a plant is dead.

Growth of the spots is usually, although not always, sharply delimited by the veins or midrib (Fig. 3, B and C). The fungus apparently does not interfere with the conductive tissue as such but does reduce the available leaf surface by a considerable amount.

Inoculations on old leaves are unsuccessful, but naturally produced spores atomized on young leaves produce recognizable infections in 5 days.

No *Pellicularia* spores were produced in artificial cultures.

Hosts and Geographical Range

The disease has been found on *Lonchocarpus utilis* only in the area between Tingo María and Pucallpa. Introduced plants of *L. urucu* at Tingo María Station have not developed infections of this disease. A wild leguminose host known as Pacae silvestre (*Inga* sp.) has been found with leaf spots with similar symptoms from which a *Rhizoctonia* resembling that from the cube spots has been isolated. It has not been possible to cross-inoculate to prove whether there is any relationship between the two diseases.

In the zone of Tingo María, somewhat similar target-spot leaf diseases caused by a strain or strains of *Pellicularia filamentosa* have been found on rubber, *Hevea brasiliensis* (H. B. K.) Muell. Arg. (7, 8), Kenaf, *Hibiscus cannabinus* L. (3), Roselle, *Hibiscus sabdariffa* L. (3), and on various varieties of bean and cowpea, *Phaseolus vulgaris* L. and *Vigna sinensis* (Torner) Savi (3, 10, 12). Comparison of the Rhizoctonia stages isolated from these hosts with that from barbasco indicates that, while the Pellicularia stages are referable to *P. filamentosa*, the strains from the above listed hosts are distinct from the strain on barbasco.

CEPHALOSPORIUM LEAF WILT

Cause

Leaf wilt is caused by a species of *Cephalosporium* that primarily attacks the veins or midrib of the leaves. A search of the literature discloses no member of this genus to which the *Cephalosporium* from barbasco can be referred. No perfect or other imperfect stage has thus far been found. Old spots on mature leaves appear to show the beginning of pycnidial development but to date no recognizable genus has been found associated. Cephalosporium fruiting continues on the outer edge of these older spots. The *Cephalosporium* is therefore described as a new species.

Cephalosporium deformans sp. nov.

Mycelium in culturis primo appressum, deindo albo-roseum sparsum, paulo granulatum cum caespitibus. Mycelium aerium ex hyphis multis parallelibus adhaerentibus,

primo hyalinis sed deinde obscuris, parce ramosis, 1.7–2.6 μ diam., indistincte septatis, saepe compositum. Conidiophora hyalina, simplicia et non septata, 1.7–2.5 μ lata ex basi, sed apicem versus gradatim attenuata, 11.9–75.0 μ longa, plerumque ad angulum rectum ex hyphis emergunt. Conidia singula hyalina, sed in massis rosea salmonis vel rubida, continua, elliptica, 8.5–17.0 × 5.1–6.8 μ, plerumque 11.9 × 5.1 μ in natura, sed in culturis paulo minora, acrogena, in capitulis globosis circum 37.5 μ diam., in aqua et aere mox dissolventibus aggregata.

Mycelium in culture[4] at first appressed, later pinkish white, sparse, somewhat granular and tufted; aerial mycelium often composed of many parallel adherent hyphae that are hyaline at first, later dark, sparsely branched, 1.7 μ to 2.6 μ in diameter with indistinct septations; conidiophores hyaline, simple, nonseptate, 1.7 μ to 2.5 μ wide at the base, tapering slightly to the tip, 11.9 μ to 75.0 μ long, mostly arising at right angles to the hyphae; conidia hyaline when viewed singly but salmon-colored to red in mass, continuous, elliptical, 8.5–17.0 μ × 5.1–6.8 μ, mostly 11.9 × 5.1 μ, in nature, slightly smaller in culture, produced acrogenously and forming globose heads, averaging 37.5 μ in diameter, which break up readily in air or water.

Cephalosporium deformans differs from most described members of this genus in having abundant production of salmon- or red-colored spores and in having a faint pink color in culture. *C. acremonium* Cda., *C. carpogenum* Ruehle, and *C. diospyri* Crandall are described as being faintly pink in culture. *C. deformans* may be differentiated from them by its larger spores. *C. acremonium*, in the later concept of this species summarized by Reddy and Holbert (9), has spores averaging 4.5 μ × 1.3 μ. The isolates with which they worked had a spore size of 3.6 μ × 1–1.8 μ and average size of 4.3 μ × 1.3 μ. *C. carpogenum* Ruehle (11) is described as having spores 4–8.5 μ × 1.4–2.8 μ. *C. diospyri* Crandall (2) is described as having spores 2.7–11.7 μ × 1.8–5.4 μ but mostly ovate ellipsoid to cylindric, 2.7–4.5 μ long.

Symptoms and Pathological Anatomy

Initial infection regularly occurs on young leaves before they reach full size. The disease first manifests itself as a tiny, slightly chlorotic spot, which on the leaf surface between veins enlarges to about 1 to 2 mm. and becomes surrounded by a pinkish to lavender halo (Fig. 4, A and B). This spot then ceases development and becomes necrotic, and spore production occurs on the under side of the leaf. Far more typically, however, the first infection is on or near the midrib or a vein (Fig. 4, C and D). This spot increases in size and develops the lavender halo, but after the fungus enters the interior of the vein or midrib the lesion rapidly elongates (Fig. 4, G and H) along this vein or midrib. On young leaves the leaf area beyond the lesion dies (Fig. 1, C and D; Fig. 4, E and F), and this necrotic area becomes indistinguishable from the primary lesion. Sporulation on the under side of the leaf occurs on the original spot in small pinpoint clusters. When slightly older leaves are attacked it is common to find large necrotic areas surrounding the primary lesion, but the entire leaf surface

[4] In the description which follows, characteristics or measurements on agar refer to growth on Difco malt agar.

beyond the lesion does not die back at once. Spore production continues for many weeks during periods of moist weather unless a primary lesion has developed on the midrib. In this case the leaflets beyond the lesion usually wither and the leaf dries and drops.

Inoculations on old leaves are unsuccessful, but spores produced in culture and in nature, atomized onto the young leaves, produced infections with typical symptoms in 7 days.

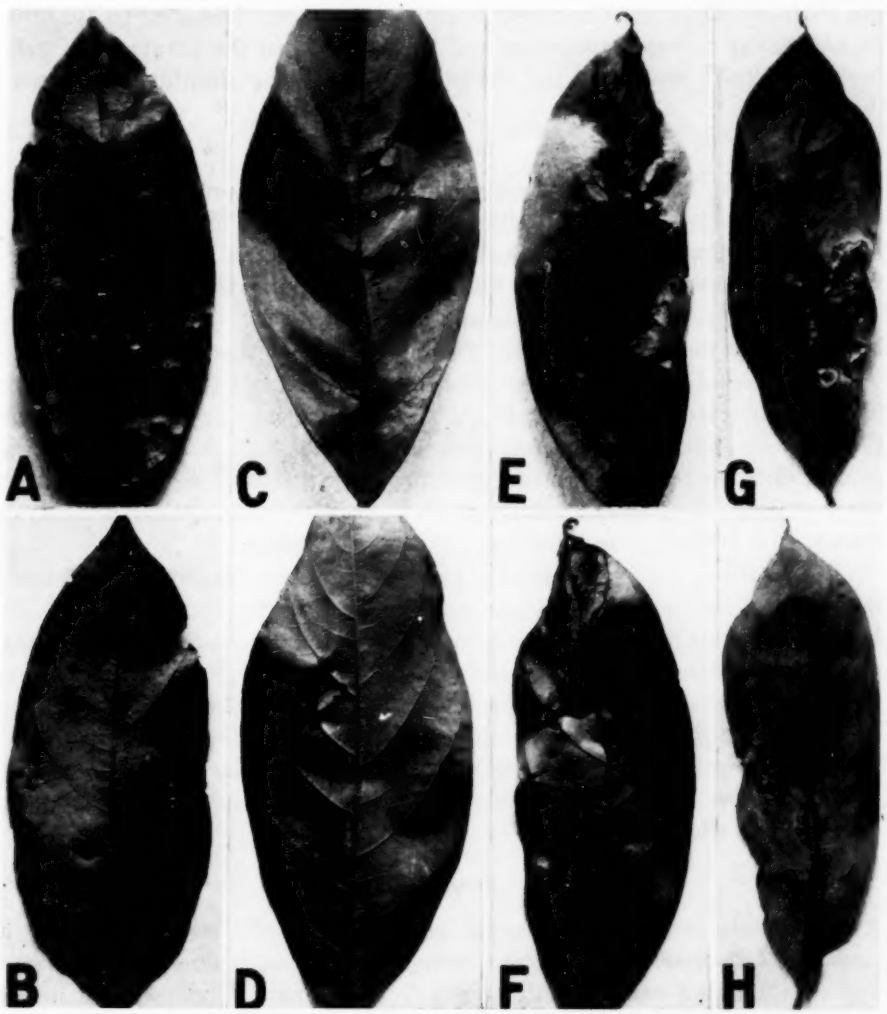

FIG. 4. *Cephalosporium deformans.* A and B, upper and lower leaf surfaces with prefruiting infections; C and D, fruiting age vein infections; E and F, showing dieback caused by vein infection; G and H, mature spot showing vein deformation.

Hosts and Geographical Range

The disease has been found only on *Lonchocarpus utilis* and thus far has been observed only on the barbasco growing in the area along the highway between Tingo María and Pucallpa.

EPIDEMIOLOGY

All three diseases are more pronounced and cause greater damage during the rainy season than in the dry season. The diseases are much more severe on new plantings established adjacent to old plantings. New plantings established early in the wet season may be considerably retarded as compared to plantings started later in the wet season and making all but their initial growth after the dry season starts. In only a few cases have the *Pellicularia* or the *Cephalosporium* diseases been found to be limiting factors in the barbasco plantings and then only when the plants were making poor growth because of soil conditions or when the plantings were heavily shaded.

CONTROL

In practice, barbasco is usually the first cultivated crop to be planted after land is cleared from forest. Isolated plantings often remain practically free from disease until the second or third year. The roots are harvested after about 3½ years. It has not been found possible to recommend sanitation where the area is replanted to barbasco. Fortunately much barbasco land is used subsequently for other crops, especially coca (*Erythroxylon coca* Lamarck). It is practical to recommend that new plantings not be made directly adjacent to old mature plantings and, in the case of the rust, the elimination of adjacent sacha barbasco. Additional experimental data on time of planting will probably result in determination of the best time of year for planting, both from the standpoint of holding disease to a minimum and also for physiological reasons.

Wide variation in susceptibility to all three diseases exists in the plantings of the native *Lonchocarpus utilis* at the Tingo María Station. It appears that this difference may be related to thickness and time of development of the cutin layer on the leaflets. It is possible, therefore, that this factor, when observed in plants selected for high rotenone content, can be used to select plants less susceptible to disease. Evidence from artificial inoculations indicates that the infection court is regularly the upper leaf surface of new leaves, with subsequent development and spore production occurring on the lower surface.

SUMMARY

Three foliage diseases affecting new and recently matured leaves of barbasco, *Lonchocarpus utilis*, are described. A rust caused by *Dicheirinia archeri* Cummins, originally described from Surinam, is widespread in the region of Iquitos, Peru, and between Tingo María and Pucallpa, Peru. A target spot disease caused by *Pellicularia filamentosa* (Pat.) Rogers and a leaf wilt caused by *Cephalosporium deformans* sp. nov. are known only from the region between Tingo María and Pucallpa, Peru. At the present time, only in occasional cases are the losses from these diseases severe enough to cause concern. Selection of plants showing resistance to disease, coupled

with high rotenone content, is contemplated in the Station's program for work on this species.

CENTRO NACIONAL DE AGRONOMÍA
SAN SALVADOR, EL SALVADOR, C. A.
IN COOPERATION WITH
U. S. DEPARTMENT OF AGRICULTURE
BELTSVILLE, MARYLAND

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THE PATHOGENIC, PHYSIOLOGICAL, AND SEROLOGICAL REACTIONS OF THE FORM SPECIES OF XANTHOMONAS TRANSLUCENS[1]

C. T. Fang, O. N. Allen, A. J. Riker, and J. G. Dickson

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INTRODUCTION

Interest in host specialization of *Xanthomonas translucens* (J. J. and R.) Dowson (4), the causal agent of bacterial stripe blight of the cereals and grasses, has been stimulated recently by divergent views regarding pathogenicity on the grass hosts. Investigations prior to 1942 have been reviewed by Hagborg (8). Because of differences in pathogenicity on wheat, rye, oats, and barley, he revised the classification and emended the species to include five closely related *formae speciales* (*f. spp.*): *hordei, secalis, undulosa, cerealis,* and *hordei-avenae.* In 1945 the variety *phlei-pratensis* was reported by Wallin and Reddy from timothy (18). A year later, Wallin found that the strains from barley, rye, wheat, and smooth brome grass were cross-inoculable; he reduced them to races of the variety *cerealis,* which he redescribed (16).

Besides pathogenicity, the organisms from different hosts were similar in morphology (13, 15, 16, 18), cultural characteristics (8, 9, 13), physiology (7, 8, 13), and serological reactions (5, 6, 8, 9). Some minor differences were reported (1, 6, 7, 15, 18), particularly with the utilization of carbon compounds, but they were too variable to give distinct differentiation.

Since it has been demonstrated that the cultures from the poured-plate method were not always reliable (19), the present studies are based upon cultures purified by single-cell technique. The specialization of this organism was considered important in relation to epidemiology on the economic hosts and of technical interest in the classification of bacterial plant pathogens.

The investigation of comparative pathogenicity of single-colony and of single-cell cultures of *X. translucens* from six hosts, together with some physiological and serological reactions, are reported in this paper.

MATERIALS AND METHODS

During the summer of 1946, thirteen cultures of *X. translucens* were obtained from various sources.[2] In addition, a culture of *X. holcicola* (Elliott) Starr and Burk. from Sudan grass was used as a control. These

[1] The authors are indebted to Mr. Eugene Herrling for preparation of the illustrations. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

[2] Dr. Hagborg of Canada and Dr. Wallin of Iowa kindly sent us some of these cultures.

cultures were single-celled to give 64 single-cell cultures. A list of the cultures with their histories is given in table 1.

Chambers' single-cell technique, improved by Wright and McCoy (20), Wright and Nakajima (21), and by others (14) was used mechanically to separate individual cells. The organisms were grown in a modified form of Medium No. 79, which is commonly employed for rhizobia. This medium, as used, had the following composition: $MgSO_4 \cdot 7H_2O$, 0.2 gm.; NaCl, 0.2 gm.; K_2HPO_4, 0.2 gm.; $CaCl_2$, 0.1 gm.; Difco yeast extract,

TABLE 1.—*Sources of Xanthomonas translucens cultures which were single-celled*

Original host	Designation of culture	Origin
Barley	2049	*X. translucens f. sp. hordei* from Hagborg.
	3055	*X. translucens f. sp. hordei-avenae* from Hagborg.
	20-1	Race 4 of Wallin, a reisolate of a barley isolate from a water-soaked streak on a barley leaf artificially inoculated.
	H-20	An isolate from a water-soaked streak on a barley leaf collected from Kanawah, Iowa, 1946.
Wheat	3045	*X. translucens f. sp. undulosa* from Hagborg.
	X-T-8	Race 2 of Wallin, formerly Hagborg's No. 618 of *X. translucens f. sp. undulosa.*
	X-T-9	Race 1 of Wallin, formerly Hagborg's No. 1236 of *X. translucens f. sp. cerealis.*
	42	Race 3 of Wallin, a reisolate from a water-soaked streak on a wheat leaf artificially inoculated with Hagborg's *f. sp. cerealis.*
Rye	10-b	Race 5 of Wallin, a reisolate of a rye isolate from a water-soaked streak on rye artificially inoculated.
Brome grass	60-1	Race 6 of Wallin, an isolate from a blackish streak on a dead brome grass leaf.
	B-126	An isolate from water-soaked streak on a brome grass leaf collected from Iowa, 1946.
Quack grass	AG-27	An isolate from water-soaked streak on a quack grass leaf collected in Madison, Wis., 1946.
Timothy	PH-1	An isolate from necrotic streak on timothy leaf collected from Hancock nursery, Wis., 1946.
Sudan grass	SA-42	A culture of *X. holcicola* from Sudan grass, Madison, Wis., 1946.

1.0 gm.; sucrose, 5.0 gm.; and distilled water, 1000 ml. The reaction was adjusted to pH 7.0. A clear agar medium was prepared by growing the organism in the liquid medium described above for 16 to 18 hr. at 28° C. and filtering it through a Seitz filter; 2 per cent agar was added to the filtrate and it was steamed until the agar was melted, then cleared by means of egg albumin and centrifugation (6).

The procedures employed in isolating and culturing a single bacterium were essentially the same as the original method, except for the following modifications developed at Notre Dame University. The micro-needle was

prepared by sealing the micro-pipette to a tiny round tip. A drop of the clear agar medium was placed in the center of a sterile cover slip and spread to form a thin, flat film of agar. After it had hardened a small drop of bacterial suspension from young active cultures, 16 to 18 hr. old, was placed at the margin of the agar film. This cover glass was inverted over the moist chamber on the microscope. The few cells were spread with the micro-needle by streaking over the surface of the agar film. Individual cells were then pulled to one side or the other by the micro-needle. They were well separated so that the colonies developing from them would not mix. A diagram of each such cover slip helped locate the micro-colonies later. The cover slip with the medium was removed from the chamber, and mounted with sterile vaseline on a sterile hanging-drop slide. The separated cells formed tiny colonies containing perhaps 100 cells. After 24 hr., transfers of the tiny colonies were made by the micro-needle to agar slants of the aforementioned unclarified medium in Durham tubes and later to ordinary agar slants. After they grew, transfers were made to the ordinary agar slants.

The original single-cell cultures of the various isolates were compared with 20 new isolations made in 1947. These new isolations are listed as follows: H-23, H-28, H-30 (barley); T-205, T-211, T-213, T-214, T-217, T-218, T-221 (wheat); SE-8, SE-10, SE-205 (rye); B-239, B-243, B-245 (smooth brome grass); AG-101, AG-102 (quack grass); and PH-2, PH-3 (timothy). These cultures were isolated and purified by the poured-plate method. Some of these were reisolated from the originals and were used in part to test the stability of the original cultures.

Pathogenicity of the cultures was compared on the different hosts in the greenhouse and in the field by two methods of inoculation: injection and spraying. The hosts used for differentiation included barley, wheat, rye, oats, timothy (*Phleum pratensis* L.), smooth brome grass (*Bromus inermis* Leyss.), and quack grass (*Agropyron repens* L.). Grasses were obtained by clonal propagation unless otherwise specified. In the greenhouse, five seedlings or young crown-shoots at the third-leaf stage of each host were inoculated with each culture compared. Field inoculations were made on rows of about 25 to 30 plants each, at various stages of plant development.

Two methods of inoculating the plants were 1) injection of a bacterial suspension into the leaf whorl at the base of the shoot with a hypodermic needle, and 2) spraying the bacterial suspension over the leaf surface without mechanical injury. A uniformly heavy bacterial suspension from 2-day-old cultures growing on nutrient dextrose agar was used for the hypodermic inoculations. Cultures grown in the nutrient broth for 2 to 3 days in a mechanical shaker were used in the leaf inoculations. The sprayed plants were incubated in the moist chamber for 48 hr. after inoculation. Plants in the field were inoculated in the afternoon and were not covered after spraying.

Both the type and the grade of infection were recorded. Three types were differentiated:

Translucence (T)–Water-soaked, translucent streak, with abundant exudate. The translucent streaks ultimately darkened. They were distinct from the following necrotic type of infection by water-soaking and abundant exudate. This appeared on the most susceptible hosts.

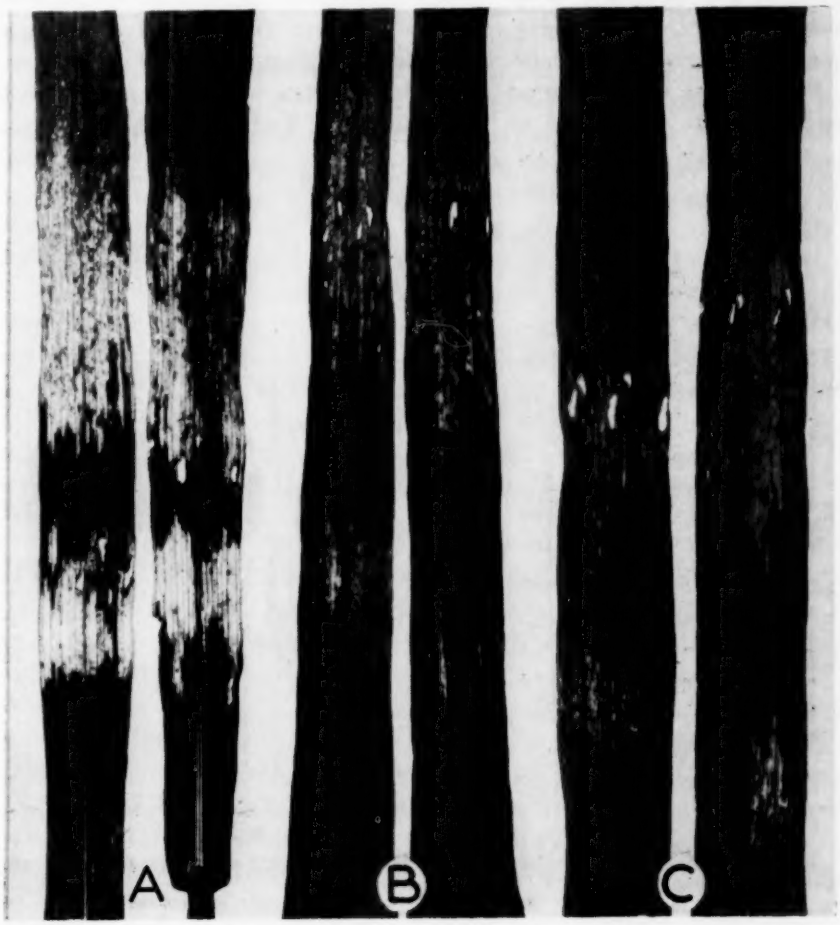

FIG. 1. Three types of infection of *Xanthomonas translucens* on smooth brome grass: A, translucence; B, necrosis; C, chlorosis.

Necrosis (N)–Brown to dark brown, sometimes black, necrosis of the leaf, no water-soaking and no exudate. This occurred on the moderately susceptible hosts.

Chlorosis (C)–Light greenish-white to yellowish-white discoloration of the leaf, no water-soaking and no exudate. This appeared on the least susceptible hosts.

Intermediate types which were common were designated by TC, TN, and so on. Three types of infection on smooth brome grass are shown in figure 1.

Difficulties were encountered in recording the grade or extent of the lesions. The size of the streak alone was unreliable because it varied with the size of the leaf. After several trials, a scale of relative size was formulated that gave comparable results on the different hosts. Five grades of infection were recorded as illustrated in figure 2. The scale, as illustrated for smooth brome grass, was applicable to all hosts except rye, in which the spread of the lesion was usually less extensive. The infection on rye was graded on a scale comparable to this. However, the grade 4 infection on rye actually corresponded more nearly to grade 3 on the other hosts.

For general bacteriological tests, the methods used were those recommended by the Committee on Bacteriological Technic of the Society of

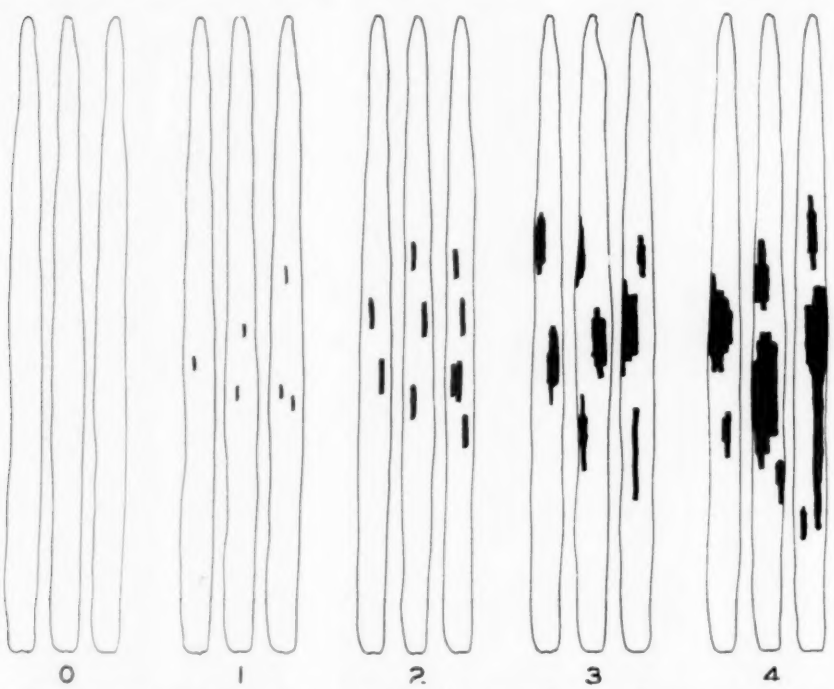

FIG. 2. Scale used for grading the infection spread of *Xanthomonas translucens* on cereals and grasses.

American Bacteriologists as given in the *Manual of Methods for Pure Culture Study of Bacteria*, Leaflet V, revised 1944 (3). Control organisms which gave positive or negative reactions respectively for each test were included. Cultures were incubated at 28° C.

For the utilization of carbon compounds, Durham fermentation tubes were used. Several media were tried including nutrient broth and synthetic nitrate medium. The most suitable one was the synthetic carbohydrate medium (3) having the following composition: $NH_4H_2PO_4$, 1.0 gm.; KCl, 0.2 gm.; $MgSO_4 \cdot 7H_2O$, 0.2 gm.; water, 1000 ml.; and carbon source, 10 gm. Difco dry yeast (0.5 gm.) extract was added because the organism failed

to grow in the above medium alone. The reaction was adjusted to pH 7.0. Brom thymol blue was the indicator. The medium was sterilized by autoclaving for 20 min. at 15 lb. pressure. Xylose, sucrose, maltose, lactose, soluble starch, and salicin were sterilized by filtering through a Seitz filter and were added aseptically. The tubes were seeded uniformly with a loop of the organism from nutrient broth, and incubated at 26° C. A change in acidity was regarded as negative if no marked change appeared in the color within 4 weeks.

For serological studies, the antiserum in rabbits was prepared by weekly intravenous injection of suspensions of living bacteria in saline (0.85 per cent), which averaged 5000 million cells per ml. Six to eight injections were made until the agglutination titer reached 1:1280 or more. The amount used was 0.5 ml. for the first injection and 1 ml. thereafter. A higher titer was not desired because the prolonged immunization decreased the specificity of the serum. As a preservative, a 1:500 dilution of merthiolate was added to the prepared serum to give the final concentration of 1:5000. The techniques of the agglutination test, the agglutinin absorption test, and the precipitin test were those given by the Manual Leaflet VIII (3), revised 1940. The gummy substances used as the antigen in the precipitin test were extracted and purified from the organism by the method used by McIntire, Peterson, and Riker for the crown-gall organism (12). The final concentration of the ethyl alcohol was about 80 per cent.

PATHOGENICITY STUDIES

Inoculation in the Greenhouse and in the Field by Injection

Infection developed rapidly after inoculation by hypodermic injection. All inoculated plants became infected; visible symptoms appeared within 3 to 4 days; data were taken after 7 days.

The reaction of 64 single-cell cultures on cereals and smooth brome grass in the greenhouse following wound inoculation by hypodermic injection indicated that cultures from different hosts were cross-pathogenic, and indistinguishable by the size and spread of lesions alone, yet they showed differences in the type of infection on the different hosts. The table showing details was omitted. Consistently the translucent type of infection was manifested by grass cultures on barley, rye, wheat, and smooth brome grass; by the wheat and rye cultures on barley, rye, and wheat; and by the barley cultures on barley and rye. *X. holcicola* from Sudan grass produced only chlorosis on these hosts. None of the cultures produced the translucent type of infection on oats, not even the cultures of Hagborg's *f. spp. cerealis* and *hordei-avenae*, which he differentiated respectively from *undulosa* and *hordei* by their greater virulence on oats (8). A repetition of these inoculations gave similar results. Varietal differences may have been responsible for this reaction on oats.

Comparisons of the different isolates from the same host revealed minor differences, but differentiation by the translucent type of infection was con-

spicuous. The frequent occurrence of the same order of variation between two replicates explained in part the differences due to experimental error. Statistical analysis of the data failed to indicate significant differences between isolates from the same host, except the barley culture, 20–1, which was different from the other cultures from barley and similar to the grass cultures. Later studies indicated that this was a barley strain found in grass.

Variations of single-cell cultures from individual isolates were, in general, less than those between them. Significant differences in pathogenicity between single-cell cultures occurred in B-126, 2049, and 10-b only. The general similarity in behavior of the single-cell cultures within an isolate warranted the use of one single-cell culture from each isolate in later experiments.

Repetition (Table 2) of the above experiments with 14 selected single-cell cultures confirmed the earlier results. Each reading given was the average of the disease reaction on five seedlings or young clones. Two new hosts, timothy and quack grass, were included in this second experiment. The reaction of quack grass was similar to that of smooth brome grass. None of the cultures produced the translucent type of infection on timothy, not even the timothy isolate (PH-1-6). If reduced size and less translucence (TC and TN) were considered as indicative of lower virulence, then the differential reaction of some of the hosts was evident. Cultures from barley were most virulent on barley, those from rye most virulent on rye, those from wheat most virulent on wheat and rye, and those from the grasses most virulent on smooth brome grass and quack grass.

Further data confirming the reaction of single-cell cultures were obtained by using the 20 new isolates made in 1947. However, the reactions of two new timothy isolates (PH-2 and PH-3) were entirely different from the others. They produced typical translucent type lesions on timothy but not on the other hosts used. This verified the work of Wallin and Reddy (18). They were different from the timothy culture (PH-1) of the previous experiments. The culture PH-1 was isolated from a necrotic streak on a timothy leaf rather than from a translucent lesion typical of the stripe blight of timothy. The similarity of the culture PH-1 to those from brome grass and quack grass suggested a form from one of these hosts.

The reisolates from the original single-cell cultures were consistent in reaction after they had passed through the different hosts. For example, the culture H-30, a reisolate of 20-1-2 from smooth brome grass, showed the same deviation from the other barley cultures noted earlier, and presented further evidence that the original culture 20-1 was not indigenous to barley. Particular attention was given to the rye and wheat cultures because they were so similar in reaction with the hosts used. The results in general indicated a relatively low virulence of the rye cultures on wheat.

With the injection method of inoculation, the results obtained from the greenhouse and field experiments agree well. In the field experiments, selected single-cell cultures were inoculated by injecting the suspension into

TABLE 2.—*Reaction of 13 selected single-cell cultures of Xanthomonas translucens inoculated by hypodermic injection into cereal and grass leaves in a greenhouse*[a]

Original host	Culture number	Grade and type of infection[b] on						
		Barley, Wis. 38	Oats, Vicland	Rye, Imperial	Wheat, Henry	Grass		
						Brome	Quack	Timothy
Quack grass	AG-27-2	1.3 T	1.8 C	2.0 TC	2.0 TC	4.0 T, TN	4.0 TN, TC	2.0 C
Timothy	PH-1-6	1.3 T	1.5 C	1.8 TC	2.0 T, TC	4.0 T	3.8 T	2.0 C, CN
Brome grass	B-126-2	1.5 T	1.5 C	2.0 T, TC	2.5 T, TC	4.0 T	4.0 T	2.0 C
	60-1-5	1.8 T	2.0 C	2.0 T	2.3 T, TC	3.8 T	3.8 T	2.0 C
Barley	20-1-2	1.0 T	2.0 C	2.0 TC	2.0 TC	4.0 T	3.5 T	2.3 C
	H-20-3	3.5 T	2.0 C	2.0 CT	2.0 C	2.0 C, NC	2.0 C	1.5 C, NC
	2049-6	2.8 T	2.0 C, NC	2.0 CT	2.0 C	2.3 NC	1.5 NC	2.0 NC
	3055-3	3.3 T	1.5 C	1.8 CT	2.0 C	2.3 C, NC	2.3 C, NC	2.0 NC
Wheat	3045-2	1.3 T	1.8 C	2.8 T	3.3 T	3.0 NC	2.3 C	1.8 C
	42-2	1.3 T	2.0 C	2.0 T	3.5 T	2.0 C	2.5 NC	1.8 C
	X-T-8-1	1.8 T	2.0 C	3.0 T	3.0 T	2.3 C, NC	2.5 C, NC	1.8 C
	X-T-9-2	1.0 T	2.0 C	2.0 T	2.5 T	2.3 C	2.5 C, NC	2.0 C
Rye	10-b-3	1.3 T	2.3 C	3.5 T	2.0 C, TC	2.8 C, NC	2.8 C	2.3 C
Sudan grass	SA-42-1[c]	1.0 NC	1.5 C, NC	1.5 C	1.5 C	2.3 C	2.5 C, NC	2.3 C

[a] Average of two replicates.
[b] The grade is illustrated in figure 2; for type, T = translucent, C = chlorotic, and N = necrotic lesion.
[c] *Xanthomonas holcicola* as control.

the plants of the central row of three-row blocks just prior to heading. The average reactions of 25 to 30 plants in each 3-ft. row are given in table 3. Although less typically translucent in some cases, the type of lesion was distinct, especially with the wheat cultures on wheat. This was comparable with natural infection on wheat leaves, where usually there were more necrotic lesions than translucent, water-soaked lesions. The spreading of the disease to the adjacent noninoculated guard rows of the blocks occurred only in the cultures producing the translucent type of infection on their respective hosts.

TABLE 3.—*Reaction of 13 selected single-cell cultures of Xanthomonas translucens inoculated by hypodermic injection on leaves of cereals and smooth brome in the field*

Original host	Culture number	Grade and type of infection[a] on				
		Barley, Wis. 38	Oats, Vicland	Rye, Imperial	Wheat, Henry	Grass, Brome
Quack grass	AG-27-2	3.5 T	3.5 C	2.0 T	3.5 TC	3.5 T
Timothy	PH-1-6	3.5 T	2.5 C	2.0 TC	3.5 TC	4.0 T
Brome grass	B-126-2	4.0 T	3.5 C	3.0 TC	3.5 TC	4.0 T
	60-1-5	3.0 TC	3.0 C	2.0 TC	3.0 TC	4.0 T
Barley	20-1-2	3.0 TC	3.0 C	3.0 TC	3.0 TC	2.5 TC
	H-20-3	4.0 T	2.5 C	2.0 TC	3.0 C	3.0 C
	2049-6	3.5 T	2.0 C	3.0 TC	3.0 C	3.0 C
	3055-3	4.0 T	2.5 C	2.5 TC	3.5 C	2.0 C
Wheat	3045-2	3.0 TC	3.0 NC	3.5 T	4.0 T	3.0 NC
	42-2	3.5 T	2.5 C	3.0 T	3.5 TC	2.5 C
	X-T-8-1	4.0 T	2.5 NC	3.5 T	3.0 TN	3.0 CN
	X-T-9-2	3.5 T	3.5 C	2.0 T	3.0 TC	2.0 CN
Rye	10-b-3	2.0 T	2.0 C	3.0 TC	3.5 TC	2.0 C
Sudan grass	SA-42-1[b]	2.0 C	2.0 C	1.0 C	2.0 C	1.5 C

[a] The grade is illustrated in figure 2; for type, T = translucent, C = chlorotic, and N = necrotic lesion.

[b] *Xanthomonas holcicola* as control.

Inoculation in the Greenhouse and in the Field by Spraying

Inoculation by the spray method gave slower development of lesions and more variation in grade of infection than with the injection method. The infection was never as extensive and the number of plants infected was more variable, especially in field experiments. The incubation period varied from 6 to 8 days, and the data were taken after 14 days.

Inoculation by spraying in greenhouse experiments gave clear-cut differentiation of the form of the organism, although infection was not extensive. The data obtained with single-cell cultures are summarized in table 4. The reading given for each replicate was obtained by averaging the disease reaction of five seedlings or clones. Barley was infected by all the cultures except the control (SA-42-1), but it was more susceptible to the barley cultures. Rye and wheat were infected by rye and wheat cultures, but only to a slight extent by the grass cultures. Smooth brome grass and quack grass

TABLE 4.—*Reaction of 13 selected single-cell cultures of Xanthomonas translucens inoculated by spraying leaves of cereals and grasses in the greenhouse*[a]

Original host	Culture number	Grade and type of infection[b] on						
		Barley, Wis. 38	Oats, Vicland	Rye, Imperial	Wheat, Henry	Grass		
						Brome[c]	Quack	Timothy
Quack grass	AG-27-2	1.5 T	0	0.5 T	0.3 T	1.4 T	1.8 T	0
Timothy	PH-1-6	1.2 T	0	0	0.2 T	2.0 T	2.0 T	0
Brome grass	B-126-2	1.5 T	0	0	0	1.8 T	1.5 T	0
	60-1-5	0.8 T	0	0.3 T	0	1.5 T	1.5 T	0
Barley	20-1-2	1.0 T	0	0	0	1.3 T	1.5 T	0
	H-20-3	3.0 T	0	0	0	0	0	0
	2049-6	2.3 T	0	0	0	0	0	0
	3055-3	2.7 T	0	0	0	0	0	0
Wheat	3045-2	1.0 T	0	0.7 T	2.2 T	0	0	0
	42-2	1.3 T	0	1.7 T	2.3 T, TN	0	0	0
	X-T-8-1	0.8 T	0	0.7 T	2.2 T, TN	0	0	0
	X-T-9-2	1.3 T	0	0.8 T	1.0 T	0	0	0
Rye	10-b-3	1.3 T	0	1.8 T	1.7 T	0	0	0
Sudan grass	SA-42-1[d]	0	0	0	0	0	0	0

[a] Average of three replicates.
[b] The grade is illustrated in figure 2; for type, T = translucent, C = chlorotic, and N = necrotic lesion.
[c] From seed.
[d] *Xanthomonas holcicola* as control.

were infected by the grass cultures only. Again none of the cultures infected oats. Cultures 20-1-2 and PH-1-6 showed the same abnormal reaction on all hosts as with the injection method. This proved conclusively that they were not indigenous to the host from which they were isolated.

Similar results appeared with the twenty new cultures in 1947 except that the rye cultures were much less virulent on wheat than the wheat cultures. As mentioned earlier, some of these cultures were reisolates from the former series and showed consistent reaction. Cultures PH-2 and PH-3 were the only cultures infecting timothy by the spray method, a result which again indicated the specificity of this form.

Field inoculation with single-cell cultures on cereals by the spray method showed even more clear-cut specialization. The plants were inoculated three times from the seedling stage to heading. The reading given for each inoculation was the average of 25 to 30 plants in each 3-ft. row. The results are summarized in table 5. Barley, very susceptible to barley cultures, was infected only slightly by the wheat and grass cultures. Varietal differences were noticed; Wisconsin Barbless (Ped. 38) was slightly more susceptible than Oderbrucker or Chevron. Rye was equally susceptible to the rye and wheat cultures, and was somewhat less susceptible to the grass cultures. Wheat was infected only by the wheat cultures. None of four varieties of oats was infected.

Because of uncontrollable environment, the field results were more variable than the greenhouse results, but still they were comparable. Consistently, if the results were negative or uncertain in the greenhouse with certain cultures and hosts, the results were also negative or uncertain in the field with these cultures and hosts.

Special Forms of the Species Based on Pathogenicity

From these studies, *X. translucens* from cereals and grasses is differentiated into five special forms (*formae speciales*) as shown in table 6. This is comparable to Hagborg's classification, except that the variety *phleipratensis* of Wallin and Reddy is included but changed to *f. sp. phleipratensis;* Hagborg's *f. spp. cerealis* and *hordei-avenae* are combined respectively with *undulosa* and *hordei* until cultures more virulent on oats are obtained to insure their separation; and the *f. sp. cerealis* is restated to include the smooth brome grass and quack grass organisms. The *f. spp. hordei, undulosa, secalis,* and *phleipratensis* maintained their original definition (8, 10, 11, 13, 15, 18), but the *f. sp. secalis* was similar to *undulosa* and produced more water-soaked infection on barley and wheat than was originally reported (13). Following is the new statement of the *f. sp. cerealis:*

Xanthomonas translucens f. sp. cerealis (Hagborg) *n. stat.*

Occurs naturally on smooth brome grass and quack grass; produces water-soaked infection on barley, rye, wheat, smooth brome grass, and quack grass following wound inoculation, but it is more virulent on grasses. Will

TABLE 5.—*Reaction of 13 selected single-cell cultures of Xanthomonas translucens inoculated by spraying the leaves of cereals in field*[a]

Original host	Culture number	Grade and type of infection[b] on									
		Barley			Oats				Rye, spring	Wheat	
		Oderbrucker	Wis. 38	Chevron	Mindo	Vieland	Bonda	Forvic		Newthatch	Henry
Quack grass	AG-27-2	0.3 TN	0	0	0	0	0	0	0	0	0
Timothy	PH-1-6	0.3 TN	1.3 TN	0	0	0	0	0	0.7 TN	0	0
Brome grass	B-126-2	0.3 TN	1.3 TN	0	0	0	0	0	0.7 TN	0	0
	60-1-5	0	0	0	0	0	0	0	0	0	0
Barley	20-1-2	0	0.3 TN	0	0	0	0	0	0	0	0
	H-20-3	2.5 T, TN	3.5 T, TN	2.5 T, TN	0	0	0	0	0	0	0
	2049-6	1.3 TN	2.7 T, TN	0.3 TN	0	0	0	0	0	0	0
	2055-3	2.7 TN, TC	3.3 TN	2.3 TN, TC	0	0	0	0	0	0	0
Wheat	3045-2	0	0	0	0	0	0	0	2.7 TN	3.3 TN	3.5 TN
	42-2	0	1.7 TC, TN	0	0	0	0	0	2.7 TN	2.5 TN	2.8 TN
	X-T-8-1	0	0	0	0	0	0	0	2.3 TN	3.3 TN	3.0 TN
	X-T-9-2	0	0.7 TN	0	0	0	0	0	0.7 TN	0.7 TN	1.5 TN
Rye	10-b-3	0	0	0	0	0	0	0	2.0 TN	0	0
Sudan grass	SA-42-1[c]	0	0	0	0	0	0	0	0	0	0

[a] Average of three replicates.
[b] The grade is illustrated in figure 2; for type, T = translucent, C = chlorotic, and N = necrotic lesion.
[c] *Xanthomonas holcicola* as control.

TABLE 6.—*Reaction of five special forms of Xanthomonas translucens resulting from injection and from spraying*

Form used	Natural host	Type[a] of infection on:						
		Barley	Rye	Wheat	Brome grass	Quack grass	Timothy	Oats
Inoculation by hypodermic injection:								
F. sp. hordei	Barley	T	T, TC	C	C, NC	C, NC	C, NC	C
F. sp. secalis	Rye	T	T	T, TC	C, NC	C, NC	C, NC	C
F. sp. undulosa	Wheat	T	T	T	C, NC	C, NC	C, NC	C, NC
F. sp. cerealis	Brome and quack grass	T	T, TC	T, TC	T	T	C, NC	C
F. sp. phlei-pratensis	Timothy	C	C	C	C, NC	NC	T	C
Inoculation by spraying:								
F. sp. hordei	Barley	T	±	0	0	0	0	0
F. sp. secalis	Rye	±	T	±	0	0	0	0
F. sp. undulosa	Wheat	±	T	T	0	0	0	0
F. sp. cerealis	Brome and quack grass	T	±	±	T	T	0	0
F. sp. phlei-pratensis	Timothy	0	0	0	0	0	T	0

[a] T = translucent type, N = necrotic type, C = chlorotic type, ± = translucent variable, and 0 = no infection.

infect smooth brome grass, quack grass, and barley by spraying, but is only slightly infectious to rye and wheat.

Five cultures (B-126-2, H-20-3, 3045-2, 10-b-3, and PH-2) representing these five forms were tested again simultaneously in the greenhouse by two methods of inoculation. The earlier results were confirmed. These five cultures were used in the serological studies.

PHYSIOLOGICAL STUDIES

Since the species were separated into five special forms by pathogenicity reactions, their bacteriological characters were examined. Extensive morphological and cultural studies were not made. More gummy substances were produced by cultures of the *f. spp. hordei* and *cerealis* than the others when grown on semisynthetic carbohydrate medium, with glucose or sucrose as the carbon source.

General Tests

The 61 single-cell cultures representing the *f. spp. hordei, undulosa, secalis,* and *cerealis* and two new isolations belonging to the *f. sp. phleipratensis* were compared. All cultures were alike, unless otherwise noted, in the following characteristics:

Nitrate reduction. No nitrate was found in the nitrate broth after 2, 5, and 9 days when tested by Trommsdorff's reagent.

Ammonia production. A moderate amount of ammonia was found in the nitrate broth after 5 days when tested by Nessler's reagent.

Indole production. No indole was detected in the tryptone (Bacto) medium after 8 days by using Kovac's test.

Liquefaction of gelatin. Liquefaction occurred after 3 days. Variation in the rate of liquefaction was found among the cultures. All showed complete liquefaction after 16 days.

Hydrolysis of starch. No hydrolysis of the starch in the nutrient agar medium with 1 per cent potato starch appeared after 7 days as tested by the plate technique.

Litmus milk. Reduction of the litmus and serum zone formation were found. The reaction after 21 days was neutral to alkaline. The rates of reduction and of serum zone formation were variable.

The methyl-red and Voges-Proskauer tests. The medium employed was as follows: Peptone (Difco) 5.0 gm.; (K_2HPO_4) 5.0 gm., and distilled water 1000 ml. The Voges-Proskauer test was made by adding 2 drops of 2 per cent ferric chloride to 5 ml. of the medium, and then 5 ml. of 10 per cent sodium hydroxide. Both the methyl-red and Voges-Proskauer tests were negative after 3 and 8 days.

Hydrogen sulfide production. Hydrogen sulfide was produced from the nutrient broth after 7 days as shown by the test strip technique.

A repetition of these trials gave similar results.

Utilization of Carbon Compounds

The acid and gas production from carbon compounds by 61 single-cell cultures was determined with a semisynthetic medium in Durham fermentation tubes. Acid was observed from glucose, sucrose, and lactose, but not from maltose. The results with mannitol and glycerol were variable. In some experiments, small amounts of acid were produced from mannitol and glycerol by wheat and rye cultures but not by others. No gas production was observed with any of the carbon compounds. However, all cultures showed strong turbidity with all the carbon compounds tested.

The above experiments were repeated four more times with 33 cultures representing five forms of *X. translucens* (consisting of 13 single-cell cultures from the above experiment and 20 new isolates of 1947). Xylose, salicin, and soluble starch were added to the list of carbon compounds. Acid reaction was induced by all cultures in a medium containing glucose, xylose, sucrose, and lactose, but not maltose and salicin. Slightly more acid and variable results were secured from glycerol by the *f. spp. undulosa* and *secalis*, and from mannitol by *f. sp. undulosa*.

In another series, the pH values of the media were measured weekly, but the results failed to show significant differences. Again no gas was observed.

SEROLOGICAL STUDIES

The antigen for immunization and agglutination was prepared from 48-hr. cultures on the semisynthetic medium for fermentation studies with 1 per cent sucrose and 1.7 per cent agar. On this medium abundant gummy substances were produced by the organism. Antisera against cultures B-126-2 (*f. sp. cerealis*), H-20-3 (*f. sp. hordei*), and 3045-2 (*f. sp. undulosa*) were prepared from three rabbits injected, respectively, with these cultures.

The cross agglutination reaction of 33 cultures representing five forms of *X. translucens* against such antisera indicated that, with the exception of the *f. sp. phleipratensis*, they were not differentiated by the cross agglutination test. Cultures of the *f. spp. hordei, cerealis, secalis,* and *undulosa* were agglutinated by such antisera to the titer of 1:640 to 1:1280. The reaction of the cultures of the *f. sp. phleipratensis* was different. The antiserum against the culture B-126-2 (*f. sp. cerealis*) agglutinated the cultures of the *f. sp. phleipratensis* to the titer of 1:160 to 1:320, while the antisera against the cultures H-20-3 (*f. sp. hordei*) and 3045-2 (*f. sp. undulosa*) agglutinated these cultures only at 1:80. No agglutination was found with *X. holcicola, X. campestris* (Pammel) Dowson, *X. phaseoli* var. *sojensis* (Hedges) Starr and Burk., and *Agrobacterium tumefaciens* (Smith and Townsend) Conn.

The lack of differentiation of the *f. spp. hordei, undulosa, secalis,* and *cerealis* by their cross agglutination reaction suggested further work with agglutinin absorption. Each of the above antisera was absorbed respectively by cultures B-126-2, H-20-3, and 3045-2, and then tested with five

forms of *X. translucens*. The results indicated that serologically the *f. sp. hordei* was different from *f. spp. undulosa, secalis,* and *cerealis* although they were similar to each other in their cross agglutination reaction.

The gummy substances produced by the organism might interfere with the antigenic pattern. The lack of differentiation by the cross agglutination reaction might be partly due to the possession of common gummy substances by all the forms. Ring tests showed that the above antisera (1:5 dilution) against the culture H-20-3 (*f. sp. hordei*) would precipitate the purified gummy substances from the cultures B-126-2 (*f. sp. cerealis*), H-20-3 (*f. sp. hordei*), 10-b-3 (*f. sp. secalis*), and 3045-2 (*f. sp. undulosa*) to the titer of 1:160 (the stock antigen prepared by adding the purified gummy substance to 0.85 per cent saline and adjusting the turbidity to tube 5 of McFarland's nephelometer). The chemical nature of the gummy substances was not determined.

In order to reduce the probable complications of the gummy substances, the above experiments were repeated by preparing the antigen for immunization and agglutination from "sugar-free" neutral, yeast-water agar medium (yeast extract 10 gm., water 1000 ml., and agar 17 gm.). The gummy substances produced on 48-hr. cultures of this medium were markedly reduced. Antisera against the cultures H-20-3 (*f. sp. hordei*), 3045-2 (*f. sp. undulosa*), B-126-2 (*f. sp. cerealis*), and PH-2 (*f. sp. phleipratensis*) were prepared from four rabbits. The antiserum for the *f. sp. phleipratensis* was added because previous experiments indicated the different behavior of this form.

The results of cross agglutination tests of five forms of *X. translucens* against these antisera are summarized in table 7. The behavior of the five forms toward the antisera for B-126-2, H-20-3, and 3045-2 confirmed the earlier results. They could be differentiated readily, however, by the antiserum for the culture PH-2 (*f. sp. phleipratensis*). The antiserum for PH-2 agglutinated its homologous cells to the titer of 1:1280 or above. It agglutinated the cells of the *f. sp. cerealis* to the titer of 1:640, but agglutinated the cells of the *f. sp. hordei* only at 1:80 or less. The *f. spp. undulosa* and *secalis* were intermediate between them, having the titer 1:160 to 1:320.

Results of agglutinin absorption experiments supported such differentiation (Table 8). Each of the above antisera was absorbed respectively by the cultures B-126-2, H-20-3, 3045-2, 10-b-3, and PH-2, and then tested with five forms of *X. translucens*. Except for the culture 10-b-3 (*f. sp. secalis*), these were the cultures used to produce the antisera. These results showed again 1) the closeness of the *f. spp. cerealis* and *phleipratensis*, 2) the similarity of the *f. spp. secalis* and *undulosa*, and 3) the greater difference of the *f. sp. hordei* from *phleipratensis* than from the other forms. Disregarding the differential behavior of the antiserum against the *f. sp. phleipratensis* (PH-2), the *f. sp. hordei* could also be differentiated from *f. spp. undulosa, secalis,* and *cerealis* by the agglutinin absorption test.

Consistently, the antisera against *f. spp. cerealis, undulosa,* and *hordei,* after having been absorbed by *f. sp. hordei,* gave considerable agglutination of the cells of the other forms (*f. spp. cerealis, undulosa,* and *secalis*). Conversely, the antiserum for the *f. sp. hordei* agglutinated the cells of the *f. sp. hordei* after being absorbed by the heterologous cells. This con-

TABLE 7.—*Cross agglutination reactions of five special forms of Xanthomonas translucens*

Organism agglutinated		Antiserum prepared for culture			
		B-126-2	H-20-3	3045-2	PH-2
F. sp. cerealis	B-126-2	+++++[a]	+++++	+++++	++++
	AG-27-2	+++++	++++	+++++	++++
	PH-1-6	+++++	+++	+++++	++++
	60-1-5	+++++	++++	+++++	++++
	20-1-2	+++++	++++	+++++	++++
	B-239	+++++	+++	+++++	++++
	B-243	+++++	+++	+++++	++++
	B-245	+++++	++++	++++	++++
	AG-101	+++++	++++	+++++	++++
	AG-102	+++++	++++	+++++	++++
	H-30	+++++	++++	+++++	++++
F. sp. hordei	H-20-3	++++	++++	+++++	+
	2049-6	+++++	+++++	+++++	+
	3055-3	++++	++++	++++	0
	H-23	++++	+++++	+++++	+
	H-28	+++++	+++++	+++++	0
F. sp. undulosa	3045-2	+++++	++++	+++++	++
	42-2	+++++	+++++	+++++	+++
	X-T-8-1	+++++	++++	+++++	++
	X-T-9-2	++++	+++	++++	++
	T-205	+++++	++++	+++++	++
	T-211	+++++	+++++	+++++	++
	T-213	+++++	++++	+++++	+++
	T-214	+++++	+++++	+++++	+++
	T-217	+++++	+++++	+++++	+++
	T-218	+++++	+++++	+++++	+++
	T-221	+++++	++++	+++++	++++
F. sp. secalis	10-b-3	++++	++++	++++	++
	SE-8	+++++	++++	++++	++
	SE-10	++++	++++	++++	++
	SE-205	++++	++++	+++++	+++
F. sp. phleipratensis	PH-2	++	0	+	+++++
	PH-3	++	+	+	+++++
X. holcicola		0	0	0	0
X. campestris		0	0	0	0
X. phascolus v. *sojensis*		0	0	0	0

[a] 0 = no agglutination at 1:80; + = agglutination at 1:80; ++ = agglutination at 1:160; +++ = agglutination at 1:320; ++++ = agglutination at 1:640; and +++++ = agglutination at 1:1280 or above.

firmed the results of the agglutinin absorption experiments mentioned earlier.

Summarizing the results of the serological studies, the five forms of *X. translucens* were differentiated into four serological types. These four

types, arranged in order of their relationship, are as follows: (a) *hordei*, (b) *undulosa* and *secalis*, (c) *cerealis*, and (d) *phleipratensis*. The *f. spp. hordei* and *phleipratensis* were the two extremes, with *undulosa-secalis* and *cerealis* intermediate. The closeness of the *f. spp. undulosa* and *secalis* serologically coincides with their similarities in pathogenicity.

TABLE 8.—*Absorption experiments of five forms of Xanthomonas translucens*

Antiserum prepared for	Absorbed by *f. spp.*	*F. spp.* agglutinated				
		cerealis (11)[a]	*hordei* (5)[a]	*undulosa* (11)[a]	*secalis* (4)[a]	*phleipratensis* (2)[a]
F. sp. cerealis (B-126-2)	*cerealis*	0[b]	0	0	0	0
	hordei	++[c]	0	++[c]	++	+
	undulosa	+	0[c]	0	0	0
	secalis[d]	+	0[c]	0[c]	0	+
	phleipratensis	++++	++++	++++	+++[c]	0
F. sp. undulosa (3045-2)	*cerealis*	0	0	0	0	0
	hordei	++[c]	0	++[c]	++	+
	undulosa	0	0	0	0	0
	secalis[d]	0	0	0[c]	0	0
	phleipratensis	+++[c]	+++[c]	++++[c]	++++	0
F. sp. hordei (H-20-3)	*cerealis*	0	++	0	0	0
	hordei	0	0	0	0	0
	undulosa	0	++	0	0	0
	secalis[d]	0	++[c]	0	0	0
	phleipratensis	++[c]	+++[c]	+++[c]	+++[c]	0
F. sp. phleipratensis (PH-2)	*cerealis*	0	0	0	0	++
	hordei	+++[c]	0	+[c]	++	+++[c]
	undulosa	+++	0	0	0	+++
	secalis[d]	++	0	0	0	+++
	phleipratensis	0	0	0	0	0

[a] The number of cultures of the form stated that were tested for these results.
[b] See footnote of table 7 for explanation of symbols.
[c] The range of agglutination titer of different cultures within the same form sometimes included an additional + at this point.
[d] Culture No. 10-b-3.

DISCUSSION

The host specialization of the cultures of *X. translucens* from cereals and grasses warrants their separation into five special forms. The importance of "water-soaked infection" following wound inoculation, emphasized by Hagborg and previous workers (8, 11, 13, 15) in the differentiation of the organism, was demonstrated. We failed, however, to obtain the marked water-soaked infection on oats to support the establishment of Hagborg's *f. spp. cerealis* and *hordei-avenae* (8). The failure of natural infections by *X. translucens* on oats to be reported so far adds evidence for the low virulence of this organism on oats.

The importance of water-soaking as a symptom is emphasized by these considerations: the cross pathogenicity of the cultures from barley, rye, wheat, smooth brome grass, and quack grass was only partial when water-soaking of tissues was used as the criterion. The production of necrosis and chlorosis, following wound inoculation, did not indicate the suscepti-

bility of the host in nature. Under the same condition, even *X. holcicola* from Sudan grass gave marked chlorosis and necrosis on the cereals and grasses tested. Moreover, a culture did not infect a host when inoculated by spraying unless water-soaked infection was obtained by injection.

Both methods of inoculation, spraying and injection, were suitable for the differentiation of the organism on the various hosts, although spraying gave better differentiation. Rapidity and certainty of infection are the chief advantages of the injection method. The organism is carried by the seed (11, 15, 17). The importance of seedling infection to the epidemiology of the disease suggests the desirability of seed inoculation studies with these five forms.

Precautions need to be taken to determine the natural host of the isolates. Isolation of an organism from a host does not necessarily mean that the organism is indigenous. This is especially true with barley which is susceptible to a number of forms of the pathogen. Another probable complication in nature is the mixed infection.

When there is a true difference in pathogenicity, Burkholder and Starr (2) believed that some other type of differences should be demonstrated. This is illustrated by our finding of the serological differences for the forms. Five forms of *X. translucens* fall into four serological types. The similarities of the *f. spp. secalis* and *undulosa* both in pathogenicity and in serological reactions suggest their combination; however, they are retained here. The failure of previous workers (5, 6, 9) to find serological differences between the forms by the cross agglutination test was partly because *f. sp. phleipratensis* was not reported until 1945. However, the absorption experiments of Elrod and Braun (6), although somewhat inconsistent, showed differences for the culture of *X. translucens* from barley, wheat, and smooth brome grass by the specifically absorbed antiserum prepared from *X. cucurbitae* and *X. translucens f. sp. cerealis*.

Our experiments failed to show any other consistent physiological difference for the forms. Since the tests used were limited in number, further studies have a good chance of revealing such differences.

SUMMARY

Comparative studies are presented here on pathogenicity with single-cell and single-colony cultures of *X. translucens* (J. J. and R.) Dowson from cereals and grasses in the greenhouse and in the field by two methods of inoculation (spraying and injection), together with those from studies of physiological and serological reactions.

Following inoculation by hypodermic injection, cultures from different hosts were cross-pathogenic, yet they showed differences in the type of infection as evidenced by three symptoms, translucence, necrosis, and chlorosis. The cross pathogenicity of the cultures was only partial when water-soaking (translucence) of the tissue was used as the criterion.

The production of the translucent type of infection following wound

inoculation was correlated with the infectivity of the cultures by the spraying method. When the bacterial suspension was sprayed on the unwounded leaves, infection occurred only on those hosts in which the translucent, water-soaked infection was obtained by injection.

From pathogenicity studies, *X. translucens* from cereals and grasses was differentiated into five special forms (*formae speciales*) as follows: *hordei* (barley), *undulosa* (wheat), *secalis* (rye), *cerealis* (smooth brome grass and quack grass), and *phleipratensis* (timothy). The *f. sp. cerealis* of Hagborg is redescribed to include the organism from the smooth brome grass and quack grass.

By combining *f. spp. undulosa* and *secalis*, these five forms can be grouped into four serological types by the cross agglutination test and the agglutinin absorption test. On the two extremes are *f. spp. hordei* and *phleipratensis* with intermediate types *undulosa-secalis* and *cerealis*. Pending further study, *f. spp. undulosa* and *secalis* are left distinct for the present because of the minor differences in pathogenicity.

No other consistent physiological differences between these five forms were found.

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DOWNY MILDEW IMMUNITY IN SPINACH

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Downy mildew (*Peronospora spinaciae*) is the most important disease of spinach (*Spinacia oleracea*) in California, where it frequently causes major damage to the crop. Losses in other regions of the United States and in other countries also are often heavy. Control by sprays or dusts has, so far, not been practical, and cultural practices apparently have little to do with the milder epidemics which occur in California. In this State, spinach is grown primarily as an early spring crop in rotation with more important row crops. Except in a few limited areas of fall production, no spinach is grown for 8 months of the year, and the crop seldom succeeds itself on the same ground.

Because there were no effective control measures, a search for resistance to downy mildew was begun in 1946. Two approaches were made to the problem: (a) attempts to select resistant plants from large populations of existing susceptible varieties, and (b) testing of previously untried material.

SOURCES OF RESISTANCE

The only published record of a search for resistance is that of Richards[1] in his detailed study of the mildew pathogen. He tested 35 varieties, representing all the known types of spinach, found no evidence of genetic resistance, and concluded that selection of resistant plants from these varieties was not possible. Since Richards apparently did not use large numbers of plants of each variety, it seemed possible that by inoculating very large populations, a few resistant individuals might be found. Such a search within the commercial varieties currently used could, if successful, materially reduce the time and labor which a program of hybridization would require. A number of commercial varieties were tested in a routine way, using about 200 or more plants of each variety. An intensive search was made in the varieties Viroflay, Prickly Winter (including Hollandia and Morse's Dark Green Prickly), and, to a smaller extent, Giant Nobel.

For testing purposes about 200 seeds were sown per flat, and the plants were inoculated at about the first true leaf stage by spraying with a water suspension of fresh spores. Initial inoculum was obtained from commercial plantings in the San Francisco Bay area and near Davis, and maintained thereafter on susceptible plants. Inoculations were made in a moist chamber in which the plants were kept from 12 to 24 hr. before removal to the greenhouse or to the outside. Infection usually appeared within 7 to 14 days; sporulation was induced whenever desired by returning the plants to the moist chamber overnight. Particular care was taken to keep the plants

[1] Richards, Mathias C. Downy mildew of spinach and its control. N. Y. (Cornell) Agr. Exp. Sta. Bul. 718. 1938.

growing vigorously, for there appeared to be a close relationship between plant vigor and mildew development. Whenever plant growth was checked, difficulty was encountered in obtaining satisfactory disease expression. This agrees with the observations of Richards.

In the large-scale inoculations during the winter of 1946–47, approximately 15,000 plants of 5 strains of Prickly Winter (Hollandia, Morse's Dark Green Prickly), 15,000 plants of 2 strains of Viroflay, and 5,000 of Giant Nobel were tested. From these, 36 plants of Viroflay, 32 of Prickly Winter, and four of Nobel appeared to possess resistance and were saved for seed production. These surviving plants of each variety were then planted together and allowed to interpollinate, each variety being kept isolated. Since spinach is dioecious, only 16 Viroflay, 13 Prickly Winter, and one Nobel plant produced seeds. Seed of each plant was saved separately and grown for testing in the fall of 1947. On inoculation, all were as severely diseased as the respective parent varieties. Thus the possibility of selecting for even a partial degree of resistance to downy mildew from the susceptible commercial varieties appears exceedingly remote.

The following 19 commercial varieties were tested and all were highly susceptible: Amsterdam Giant, Bloomsdale (Long Standing), Bloomsdale Savoy, Broadleaved Prickly, Deventer Green, El-De-Es, Hollandia, Juliana, King of Denmark, Morse's Dark Green Prickly, Giant Nobel, Old Dominion, Prickly Winter, Prickly Winter (original strain), Supra, Utrecht Winter, Viking, Virginia Savoy, and Viroflay.

After the above testing had been largely completed, 9 seed lots from the Division of Plant Exploration and Introduction, U. S. Department of Agriculture, became available for testing. From these 9 lots (P. I. 140,461 to 140,469 inclusive), 2 lots (P. I. 140,464 and 140,467) collected in Iran were found to be mixtures of susceptible and resistant plants. The remaining lots were susceptible. The plants of lot P. I. 140,464 were lost, but those of the second Iranian lot were saved for hybridizing. Neither lot, however, was suitable for cultivation because of small, thin leaves of varying shape (Fig. 1), very high oxalic acid content, and early bolting habit.

Disease-free plants of P. I. 140,467 were then interplanted in isolation plots with plants of Prickly Winter and Viroflay. Male plants of P. I. 140,467 were removed before anthesis, and seed was saved from individual female plants of this lot in order to secure genetic data. A good seed yield was obtained, indicating a high degree of cross-fertility. Since the commercial varieties were genetically susceptible, no attempt was made to use individual plants as pollen parents.

Inoculation and inspection of plants of the parent and hybrid lines revealed only two classes of plants—badly diseased, and clean—with no intermediate levels of resistance evident. Careful macroscopic examination of disease-free plants failed to reveal any trace of fungus penetration or development. The freedom from disease, then, can be considered true immunity, rather than high-level resistance.

The consistent reaction of the immune stocks during the winters of 1947–48 and 1948–49 has not suggested the presence of any physiologic strains of the fungus in the Davis areas, although there was ample opportunity for infection from natural sources throughout the course of the experiments.

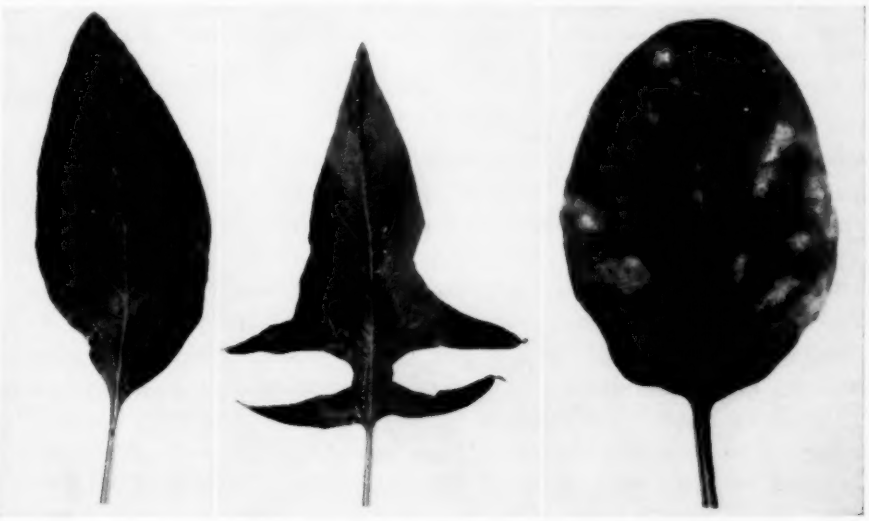

FIG. 1. Left and center, young leaves from two downy-mildew-immune spinach plants of P. I. 140467; right, infected leaf of the susceptible Viroflay variety. All three were inoculated at the same time.

INHERITANCE OF IMMUNITY

Because of the predominantly dioecious habit of spinach, self-pollination is not normally possible. Inheritance data, therefore, have been secured on the F_1 between immune and susceptible plants and on the first backcross of immune F_1 plants to the susceptible types.

Assuming a single dominant gene for immunity, in the F_1 generation of the cross between the immune and susceptible varieties, classes of progenies would be expected, (a) uniformly immune, and (b) segregating in a 1:1 ratio. The former would result from a homozygous, and the latter from a heterozygous immune plant, each crossed with the homozygous susceptible commercial parent. In the backcross of immune F_1 segregates with the susceptible parent variety, all populations should segregate in a 1:1 ratio.

The results (Table 1) show a very good agreement between the ratios obtained and those expected. The complete susceptibility of C1 indicates the supposedly immune parent to have been a susceptible escape. The two Viroflay lots included were checks for BC1, BC2, BC3, C6, and C7. Check lots of at least 1 flat (approximately 200 plants) were inoculated for all tests and were 100 per cent diseased before the experiment terminated. Occasionally, infection was not complete on the check after inoculation, and in these cases both the check and accompanying progenies for testing were

re-inoculated. Additional crosses have not been tested for inheritance data since the data shown were considered adequate.

On the basis of results obtained, immunity from downy mildew in spinach is inherited as a single dominant gene.

TABLE 1.—*Segregation for susceptibility and immunity from downy mildew. The letter C represents F_1 progenies of immune × susceptible parents; BC represents the first backcross of immune F_1 segregates × susceptible plants*

Seed	Number healthy		Number susceptible		Total	χ^2	P
	Obtained	Expected	Obtained	Expected			
Class I, homozygous susceptible							
Cl_a	0	0	313	313	313		
Viroflay (lot 1)	0	0	278	278	278		
Do (lot 2)	0	0	300	300	300		
	0	0	891	891	891		
Class II, segregating							
C2	710	727.5	745	727.5	1455	0.841	0.30–0.50
C3	185	186.5	188	186.5	373	0.024	0.80–0.90
BC1	26	29.0	32	29.0	58	0.667	0.30–0.50
BC2	41	40.0	39	40.0	80	0.050	0.80–0.90
BC3	6	7.5	9	7.5	15	0.600	0.30–0.50
	968	990.5	1013	990.5	1981	1.022	0.30–0.50
Class III, homozygous immune							
C4	230	230	0	0	230		
C5	48	48	0	0	48		
C6	140	140	0	0	140		
C7	73	73	0	0	73		
	491	491	0	0	491		

[a] The uniform susceptibility of C1 indicates that the supposedly immune parent was a susceptible escape.

SUMMARY

In a search for resistance to downy mildew of spinach, all commercial varieties tested were uniformly highly susceptible. From large populations of three commercial varieties inoculated with the fungus, it was impossible to find any degree of resistance whatsoever.

Immunity was found in two uncultivated varieties. Results obtained from the first cross, between immune plants of unknown genetic constitution and susceptible commercial varieties, and from immune F_1 segregates backcrossed to the susceptible types, show immunity to be inherited as a single dominant gene.

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GENETICS OF RESISTANCE TO THE COMMON BEAN MOSAIC VIRUS (BEAN VIRUS 1) IN THE BEAN (PHASEOLUS VULGARIS L.)[1]

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INTRODUCTION

The inheritance of resistance to the common bean mosaic virus (bean virus 1) has been a puzzle for almost 25 years. Most reports of experimental tests with it cover the results of crosses between only two or three varieties. Some of the data are seemingly contradictory.

McRostie in 1921 (4) reported the results of crosses between the resistant variety Robust and the two susceptible varieties Flat Marrow and Selection B. The F_2 and F_3 data suggested that susceptibility is governed by two dominant complementary factors, and that the degree of reaction to the virus depended upon the number of dominant factors involved.

Pierce (6) studied the inheritance of resistance to the virus in crosses between the susceptible variety Refugee Green and the resistant varieties Corbett Refugee (and its derivatives Wisconsin Refugee and Idaho Refugee), Great Northern U. I. No. 1, and Robust. The results obtained in the F_1 and F_2 generations showed that the resistance of Corbett Refugee and its two derivatives was dominant, while that of Great Northern U. I. No. 1 and Robust was recessive. The results, however, were not interpreted on a factorial basis because of the differences obtained in certain reciprocal crosses.

Parker (5) made an extensive study of reciprocal crosses between Stringless Green Refugee and Michigan Robust, carrying the tests to the F_3 generation. The F_1, F_2, and F_3 progenies generally resembled the maternal parent in the original mating, suggesting a cytoplasmic influence. The susceptible × resistant cross, however, showed a slight but gradual increase in the proportion of resistant plants from generation to generation, while the resistant × susceptible cross showed a change in the opposite direction. This convergence of the results from reciprocal crosses was interpreted as due to genic control but with delayed expression of the gene action.

Wade and Andrus (7) reported the results of crosses between the tolerant variety Stringless Black Valentine and the resistant variety U.S. No. 5 Refugee, under conditions of natural field transmission. The F_2 and F_3

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data indicated that the resistance of U.S. No. 5 Refugee was governed by a single dominant gene.

More recently, Grogan and Walker (2) developed the approach-graft inoculation technic to distinguish between varieties with the Corbett Refugee type of resistance and those which have the Robust type of resistance. The former varieties showed top-necrosis and black-root symptoms, while the latter varieties remained healthy.

The present work was started in 1947 to determine the genetic basis of resistance to bean virus 1. The investigations have progressed to the point where certain definite conclusions can now be stated.[3]

MATERIALS AND METHODS

Four varieties of beans were used in the present work, namely, Keeney's Stringless Green Refugee (susceptible), U.S. No. 5 Refugee, Idaho Refugee, and Robust (resistant). Both U.S. No. 5 Refugee and Idaho Refugee derive their resistance from the variety Corbett Refugee which was developed by R. D. Corbett from a single mosaic-resistant plant found in a heavily infected stand of Refugee green beans (6). Robust was developed by selection from a lot of commercial pea-beans containing mosaic in 1918 (1).

Crosses were made in all possible combinations between disease-free plants both in the greenhouse and in the field. The parental plants were grown in the field under a $20 \times 15 \times 7$-ft. cage, covered with cheesecloth in order to avoid insect transmission of the virus from near-by sweetclover and alfalfa plants.

The F_1 seeds were planted in the field under the cheesecloth cage and the F_2 seeds from each plant were harvested separately. The F_2 plants were grown and tested for virus reaction in the greenhouse. Throughout the period of inoculation the temperature was kept at 75 to 80° F. and the greenhouse was maintained free or almost free of insects by frequent dusting with 3 per cent DDT and spraying with 1:1000 Blot (active ingredient hexaethyltetraphosphate). A row of each of the parental varieties was grown among the lots of F_2 plants and inoculated at the same time as a check. The F_3 seeds were taken from F_2 plants which were kept disease-free, and the F_3 families were grown separately in the greenhouse for inoculation.

The Zaumeyer strain of bean virus 1, kindly made available by Dr. J. C. Walker, was used for inoculation. The virus culture was maintained in the greenhouse in Stringless Green Refugee plants grown from virus-free seed. For the most part the ordinary method of rub-inoculation was employed. In certain F_2 lots (to be discussed later) the approach-graft inoculation method was used.

[3] The writer gratefully acknowledges the constructive advice and criticism of Dr. R. A. Brink throughout the investigations and during the preparation of the manuscript. He is indebted to Dr. J. C. Walker for his interest and encouragement. Thanks must go to Eugene Herrling for preparing the illustrations.

Specimens from various parts of the grafted plants were collected for histological studies. They were prefixed in Carnoy's fluid, fixed in Karpechenko's solution, and imbedded by the tertiary butyl alcohol method. The specimens were sectioned at a thickness of 13μ and then stained with Delafield's haematoxylin and safranin combination.

EXPERIMENTAL RESULTS

The F_2 Generation

Crosses that were tested in the F_2 generation include the following: Stringless Green Refugee × U.S. No. 5 Refugee, and reciprocal; Stringless Green Refugee × Idaho Refugee, and reciprocal; Stringless Green Refugee × Robust, and reciprocal; Robust × U.S. No. 5 Refugee; Idaho Refugee × Robust. The progenies of F_1 plants in each cross were first tested separately. Since different tests with a particular cross gave similar results, the F_2 data from comparable families were later pooled.

Table 1 shows the F_2 distributions from crosses between susceptible × resistant varieties following rub-inoculation.

TABLE 1.—*F_2 distribution from crosses between susceptible × resistant varieties following rub-inoculation*

Matings	F_2 plants			χ^2	P. range
	Healthy	Diseased	Total		
Stringless Green Refugee × U.S. No. 5 Refugee	630	219	849	0.266(3:1)	0.70–0.50
Stringless Green Refugee × Idaho Refugee	409	150	559	0.859(3:1)	0.50–0.30
Stringless Green Refugee × Robust	195	547	742	0.650(1:3)	0.50–0.30

The F_2 plants from crosses involving either U.S. No. 5 Refugee or Idaho Refugee as the resistant parents are distributed in a ratio of 3 resistant : 1 diseased. This indicates a single dominant gene governing resistance in these two varieties. On the other hand, the cross Stringless Green Refugee × Robust gave an F_2 distribution of 1 resistant : 3 diseased, indicating that resistance in this case is governed by a single recessive gene.

The F_2 distributions from the reciprocals of the above crosses are given in table 2. It is apparent that there is no difference between a given cross and its reciprocal. The possibility of a cytoplasmic influence is thus excluded.

The establishment of two genetically different types of resistance leads to the question as to how these classes behave when crossed with each other. Table 3 shows the F_2 distributions from crosses between Robust on the one hand, and U.S. No. 5 Refugee and Idaho Refugee on the other.

Following rub-inoculation a ratio of 13 resistant : 3 diseased plants was observed. These data support the hypothesis of two independent

TABLE 2.—*F_2 distribution from crosses between resistant × susceptible varieties following rub-inoculation*

Matings	F_2 plants			χ^2	P. range
	Healthy	Diseased	Total		
U.S. No. 5 Refugee × Stringless Green Refugee	304	107	411	0.158(3:1)	0.70–0.50
Idaho Refugee × Stringless Green Refugee	474	161	635	0.018(3:1)	0.90–0.80
Robust × Stringless Green Refugee	212	611	823	0.195(1:3)	0.70–0.50

genes accounting for the difference in breeding behavior between the two types of resistance. In accordance with this ratio, then, there should be two classes of resistant plants which are indistinguishable by rub-inoculation. An attempt was made to identify these two classes by applying the technic of approach-graft inoculation. Plants of three types were distinguishable by this method: 1) Top-necrotic plants, or the U.S. No. 5 and Idaho Refugee type of reaction (Fig. 1); 2) healthy plants, or the Robust type of reaction; and 3) mosaic plants, or Stringless Green Refugee type of reaction.

As shown in the second part of table 3, the distribution of these three types fits a ratio of 9 necrotic:4 healthy:3 mosaic. The class of 13 resistant plants obtained by rub-inoculation is now broken down into nine of the Corbett Refugee type and four of the Robust type.

TABLE 3.—*F_2 distributions from crosses between two resistant types*

F_1 parent		F_2 Plants tested	F_2 segregation		$\chi^2(13:3)$	P. range
			Healthy	Diseased		
Rub-inoculation technique used						
Robust × U.S. No. 5 Refugee	−1	84	62	22	13.047	0.01
	−2	80	65	15	0.000	0.99
	−3	82	66	16	0.000	0.99
	−4	142	115	27	0.000	0.99
	Pooled	411	328	83	0.320	0.70–0.50
Total		799	636	163	1.282	0.30–0.20
Idaho Refugee × Robust	−1	143	117	26	0.000	0.99
	−2	170	134	36	0.799	0.50–0.30
	−3	209	166	43	0.398	0.70–0.50
	Pooled	667	537	130	0.300	0.70–0.50
Total		1189	954	235	0.862	0.50–0.30

F_1 parent	F_2 Plants tested	F_2 segregation			$\chi^2(9:4:3)$	P. range
		Top-necrotic	Healthy	Mottle		
Approach-graft inoculation technique used						
Robust × U.S. No. 5 Refugee	103	57	26	20	0.070	0.98–0.95
Idaho Refugee × Robust	150	83	40	27	0.326	0.90–0.80

FIG. 1. Top necrosis following approach-graft inoculation. Right, mosaic symptoms on a Stringless Green Refugee plant previously rub-inoculated. Left, top necrosis on an F_2 plant from the cross Robust × U.S. No. 5 Refugee.

The F_3 Generation

The crosses which were followed through to the F_3 generation are 1) Stringless Green Refugee × Robust, and 2) Robust × U.S. No. 5 Refugee. Table 4 gives the summarized data of the cross Stringless Green Refugee ×

TABLE 4.—*Classes of F_2 families based on F_3 breeding behavior of the cross (Stringless Green Refugee × Robust)*

Types of families	No. of families		Total individuals		χ^2	P. range
	Observed	Expected	H	D		
Healthy	9	11	126	.....		
1H : 3D	23	23	107	309	0.080	0.80–0.70
Diseased	13	11	.....	224		
Total	45	45				
χ^2(1:2:1)	0.728					
P. range	0.70–0.50					

Robust. The F_3 families show a distribution of 1 healthy : 2 segregating (1H : 3D) : 1 diseased. This offers further support to the conclusion based on the F_2 results that the resistance of Robust is governed by a single recessive gene.

The summarized data from the cross Robust × U.S. No. 5 Refugee are given in table 5. The F_3 families show the following distribution: 7

healthy; 4 segregating (13H : 3D); 2 segregating (3H : 1D); 2 segregating (1H : 3D); 1 diseased.

These results can be accounted for on the basis of two independently inherited genes governing resistance to the common bean mosaic virus.

TABLE 5.—*Classes of F_2 families based on F_3 breeding behavior of the cross (Robust × U. S. No. 5 Refugee)*

Types of families	No. of families		Total individuals		χ^2	P. range
	Observed	Expected	H	D		
Healthy	36	40	687	0		
13H : 3D	22	23	482	109	0.025	0.90–0.80
3H : 1D	15	11	253	96	1.106	0.30–0.20
1H : 3D	13	11	81	220	0.537	0.50–0.30
Diseased	5	6	1	126		
Total	91	91				

χ^2(7:4:2:2:1) 2.427
P. range 0.70–0.50

The Factorial Basis of Resistance

It is now established that resistance to the virus is governed by a single dominant gene in some varieties and by a single recessive gene in certain other varieties. The various combinations between the two gene pairs lead to different symptom expressions, as shown in the F_2 and F_3 of the crosses between strains differing in the two types of resistance.

The following factorial scheme explains the above results. A dominant gene *A* is essential for virus invasion and the development of mosiac symptoms. Another dominant gene *I* acts to prevent the expression of mosiac symptoms when present together with the basic gene *A*, thus being inhibitory in its action. Plants possessing this combination are resistant to the virus introduced by rub-inoculation. If the virus is continuously supplied by graft inoculation, however, the inhibitor gene *I* conditions top necrosis, the

TABLE 6.—*The genetic constitution, response to bean virus 1, and the breeding behavior of the four varieties of beans used in the present work*

Variety	Genotype	Symptoms		Genetical behavior
		Rub-inoc.	Graft-inoc.	
Stringless Green Refugee	*AAii*	Mottle	Mottle	
U.S. No. 5 Refugee	*AAII*	Resistant	Top-necrotic	Dominant
Idaho Refugee	*AAII*	Resistant	Top-necrotic	Dominant
Robust	*aaii*	Resistant	Resistant	Recessive

nature of which will be discussed later. The other possible combinations of these two genes are *ai* and *aI*. Plants of such genotypes are resistant because of the absence of the basic gene *A*, and do not show top necrosis following graft inoculation.

The genetic constitution of the four varieties used in this work, their response to the virus, and their genetical behavior are summarized in table 6. It is possible to explain the F_2 and F_3 ratios obtained from crosses between the susceptible variety and the three resistant varieties on the basis of a one-gene difference. Crosses between the resistant types segregate on a two-gene difference basis, and the manifestations of the different gene combinations depend upon the method of inoculation, as presented diagrammatically as follows:

P_1	F_1	F_2	Symptoms	
			Graft-inoc.	Rub-inoc.
		9 *AI*	9 necrotic	
aaii (Robust)		3 *aI*		13 resistant
×	*AaIi*		4 resistant	
AAII (U.S. No. 5		1 *ai*		
Refugee)		3 *Ai*	3 mottle	3 mottle

The distribution of F_3 families from the cross Robust × U.S. No. 5 Refugee can be interpreted accordingly.

The Nature of Resistance

The present observations show that where the inhibitor gene *I* is present together with the basic gene *A*, the plant shows top necrosis following graft inoculation. Even by rub-inoculation a certain number of plants show black root symptoms but are classified as resistant.

This type of reaction was studied histologically in order to determine the role of the inhibitor gene. Cross sections were made in various parts of the grafted plants, namely, at, above, and below the graft-union. Microscopic examination of the sections showed the following features:

1. Sections through the graft-union of a necrotic plant always show cell necrosis, starting at the point of contact and extending laterally in the vascular tissue. The necrosis is confined to the phloem tissue, resulting in a darkly staining ring of collapsed cells (Fig. 2, A). Parenchyma cells of the cortex across the graft-union usually show necrosis along definite paths.

2. Sections of the necrotic plant from various parts above or below the graft-union region indicate that necrosis advances upwards and downwards in the stem. Figure 2B shows that necrosis is confined to the vascular tissues, particularly in the phloem, and rarely extends into the medullary ray cells. It is not unusual to find individual xylem vessels or a whole vascular bundle plugged with darkly staining materials.

When the advancing necrosis reaches the growing point and the root system of the plant, the latter ceases to grow and dies. These symptoms are identical with the disease described as black root, which Grogan and Walker

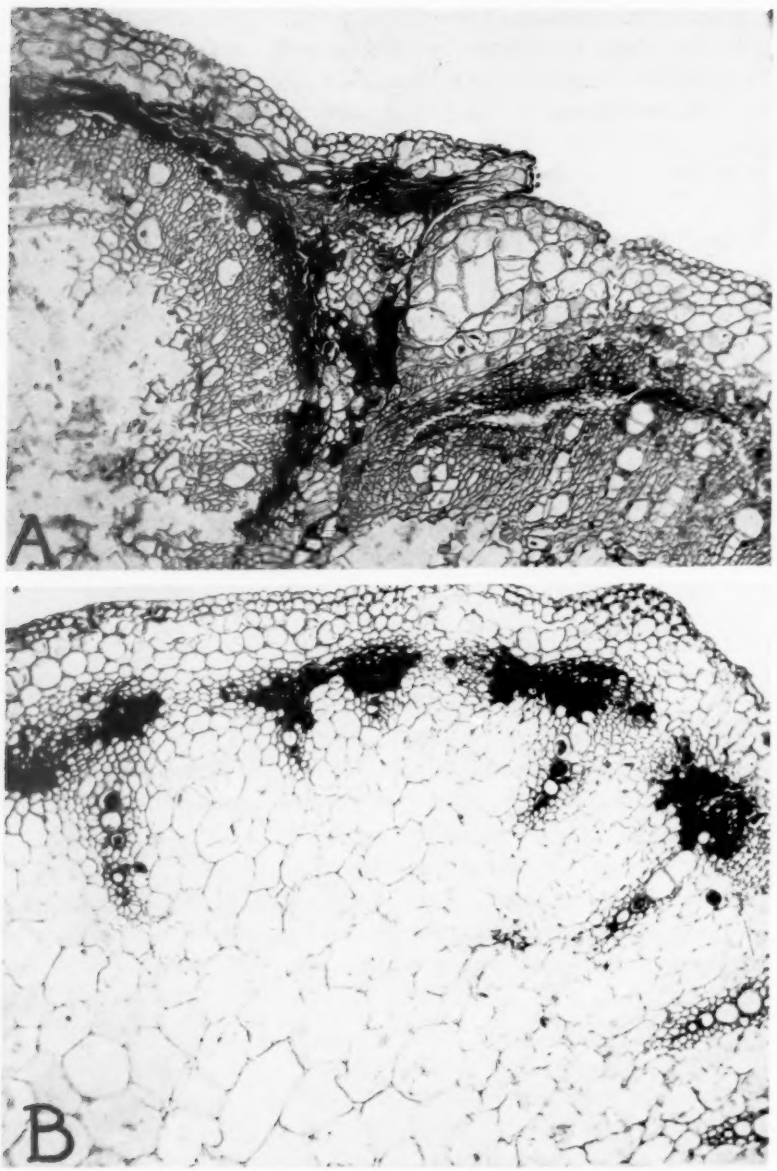

FIG. 2. Cross section of a grafted necrotic plant, (A) (right) through the graft-union, where necrosis is confined to the connecting parenchyma cells and the phloem tissue of the necrotic plant; and (B) 2½ in. above, showing necrosis still confined to the phloem tissue. A few xylem vessels are plugged with darkly staining material.

(2) demonstrated as due to bean virus 1 infecting varieties with the Corbett Refugee type of resistance. Likewise, the histological observations described here are essentially similar to those reported by Jenkins (3) on the black root affecting snap beans.

3. Comparable sections of the grafted mottled and resistant plants did not show any sign of cell necrosis. In both cases the plant continues to grow, either developing mosaic symptoms or remaining healthy, depending upon its genetic constitution.

DISCUSSION

The inheritance of resistance to the common bean mosiac virus may be interpreted in terms of two gene pairs exhibiting dominant and recessive epistasis, respectively. The inhibitor gene *I* is effective only in the presence of the basic gene *A*, while the recessive gene *a* brings about resistance either in combination with *I* or its allele *i*. Gene *A* can be thought of as providing the basic requirements for virus multiplication, thus rendering the tissues susceptible. Together with the gene *i* it permits the entry and advance of the virus, apparently in the phloem, and results in mosaic symptom expression.

The inhibitor gene *I*, on the other hand, conditions a hypersensitive reaction by producing certain substances which, in the presence of the active virus, lead to cell necrosis without suppressing the action of gene *A* itself. The necrotic cell is unfavorable for the establishment and survival of the virus and hinders its further progress. The immediate result, therefore, is checking the spread of the small amount of virus inoculum introduced by rubbing or natural field infection. But, when the virus is continuously supplied by means of approach-graft inoculation, the large quantities of virus particles overcome the defense mechanism of the cells and move to neighboring cells, leaving necrosis in their trail. When the virus reaches the vascular tissues, systemic necrosis takes place and top necrosis follows.

It is understandable, therefore, why U.S. No. 5 Refugee and Idaho Refugee (and other varieties with resistance derived from Corbett Refugee) are considered only as field resistant varieties (2). The incidence of black root symptoms observed in such varieties, following rub-inoculation or occurring naturally in the field, can be attributed to the virus inoculum escaping into the vascular tissue and causing systemic necrosis. Otherwise when viruliferous aphids feed on the leaves, the small amounts of inoculum introduced are checked in small localized areas which sometimes appear as necrotic spots. Grogan and Walker (2) correctly attributed this as "possibly based on hypersensitive reaction of the tissue to infection." They further stated that "In most cases this results in a small local necrotic area or fleck in which the virus is usually inactivated, while in a very few cases systemic necrosis and black root occur."

The present findings make it possible to classify resistance to bean virus 1 in the bean into two categories:

1. Dominant resistance, present in the varieties derived from Corbett Refugee.

2. Recessive resistance, present in Robust and possibly Michelite which originated from the pea-bean. The series of Great Northern U. I. varieties

also could be placed in this group. Although the latter varieties were not included in the present study, it has been shown previously that one of them possesses the same type of resistance as Robust, *i.e.*, Great Northern U. I. No. 1.

SUMMARY

Crosses were made between the susceptible variety Stringless Green Refugee and the three resistant varieties U.S. No. 5 Refugee, Idaho Refugee, and Robust to determine the inheritance of resistance to the common bean mosaic virus (bean virus 1). The results obtained from the various crosses are summarized in the following:

1. The crosses Stringless Green Refugee × U.S. No. 5 Refugee and Stringless Green Refugee × Idaho Refugee showed an F_2 ratio of 3 resistant: 1 susceptible, following rub-inoculation. There were no differences between the reciprocal matings. This suggested a one-dominant gene difference controlling the resistance of these varieties.

2. Crosses between Stringless Green Refugee and Robust yielded an F_2 ratio of 1 resistant: 3 susceptible, following rub-inoculation. There were no differences between the reciprocal crosses. F_3 families from this cross showed a distribution of 1 healthy: 2 segregating (1H: 3D): 1 diseased. Therefore, resistance of Robust is governed by a single recessive gene difference.

3. Crosses between the two types of resistant varieties gave an F_2 ratio of 13 resistant: 3 susceptible, following rub-inoculation. By using the approach-graft inoculation the above ratio was broken down into 9 necrotic (U. S. No. 5 Refugee and Idaho Refugee type of reaction): 4 healthy (Robust reaction): 3 mottled (Stringless Green Refugee reaction). The F_3 families from the cross Robust × U.S. No. 5 Refugee showed a distribution of 7 healthy: 4 segregating (13H: 3D): 2 segregating (3H: 1D): 2 segregating (1H: 3D): 1 diseased. These data give evidence of two pairs of independently inherited gene differences, with dominant and recessive epistasis.

A factorial scheme which explains the above results is presented as follows. A dominant gene *A* is required for virus infection, rendering the tissues susceptible. Another dominant gene *I*, when present with the basic gene *A*, inhibits symptom expression following rub-inoculation or natural field infection, and conditions top necrosis when there is a continuous supply of virus inoculum (following approach-graft inoculation). In the presence of gene *a* (acting as a recessive epistatic), the plant becomes resistant to both mosaic symptoms and top necrosis.

On this basis, the genotypes of the four varieties used are: Stringless Green Refugee, *AAii* (susceptible); U. S. No. 5 Refugee and Idaho Refugee, *AAII* (field resistant, top-necrotic); Robust, *aaii* (resistant, no top necrosis). The genetical behavior of the various crosses is interpreted accordingly.

Histological examination of the necrotic plants showed that systemic necrosis is largely confined to the phloem tissue, while both mottled and healthy plants had no signs of necrosis.

The necrotic reaction following graft-inoculation is conditioned by gene *I* only in the presence of *A*, most likely through a hypersensitive mechanism. Plants with the genotypes *aaI-*, *aaii*, or *A-ii* do not show this reaction. The first two genotypes remain healthy while the third develops mosaic symptoms.

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INFLUENCE OF NUTRITIONAL AND ENVIRONMENTAL FACTORS UPON ASEXUAL REPRODUCTION OF CHOANEPHORA CUCURBITARUM IN CULTURE[1]

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(Accepted for publication August 10, 1949)

In nature *Choanephora cucurbitarum* (B. and Rav.) Thaxter causes a rapid rot of the flowers and young fruit of certain plants, especially the cucurbits. The fact that conidia are abundant on the decayed parts only in the early morning is evidence that they are produced during the night and disseminated during the day. The rapidity of the sporulation cycle is no doubt an important contributing factor to its wide distribution and to the importance of the fungus as a pathogen in certain regions.

Under favorable cultural conditions this fungus produces two types of asexual spores; those formed in sporangia typical of the Mucorales, and the conidia formed in large heads. Wolf (11) states that only the conidial stage has been reported from nature, but more recently Palm and Jochems (6) reported finding the sporangial stage occasionally on *Amaranthus blitum* in Sumatra. Sinha (7, 8) also observed sporangia on rotting plants of *Capsicum* and *Colocasia antiquorum* in India. Illustrations of these two spore stages are given by Fitzpatrick (4) and by Wolf and Wolf (12).

The taxonomy of the fungus has been discussed by Thaxter (9). Wolf (11) made brief studies of its morphology and development in culture and reported that one isolate produced only sporangia when cultured in continuous darkness, while another "strain" produced a few sporangia and conidia in total darkness. His fungus sporulated on squash tissue in a moist chamber, but failed to do so on tubes of various media. He concluded that light is not the only factor influencing the formation of sporangia and conidia. Wolf (11) found zygospores in only one set of cultures made directly from natural material, while Sinha (7) frequently obtained zygospores in culture.

The first extensive experiments on the physiology of this fungus were conducted by Christenberry (3), who studied the effects of light of various wave lengths and periods of exposure upon asexual reproduction. In daylight the greatest numbers of conidial heads were formed in alternating 12 hr. of light and 12 hr. of darkness. Both conidia and sporangia were formed in total darkness. Using colored filters he found that red-yellow light was the most effective portion of the spectrum.

A number of the above observations of Wolf and Christenberry have been verified by the present investigations, allowing for variation which might be attributed to differences in isolates. The chief difference seems to lie in the ability of certain isolates to produce some conidia in continuous total darkness while others fail to do so.

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The purpose of this paper is to report the results of detailed experiments designed to determine the effects of various nutritional and environmental factors upon the asexual reproduction, particularly the production of conidia, of *Choanephora cucurbitarum*. A large portion of the paper deals with the action of light and its influence upon conidium formation.

MATERIALS AND METHODS

The isolate used throughout these experiments was obtained from a diseased squash flower at Morgantown, West Virginia. A second isolate, obtained through the courtesy of Dr. F. T. Wolf, was used only for comparison in some of the light experiments.

Preliminary tests were conducted to select a medium which would give abundant sporulation without excessive mycelial growth. The medium finally chosen and used in all of the experiments with temperature, light, and aeration had the following composition: glucose, 2 gm.; asparagine, 1 gm.; KH_2PO_4, 1 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; Fe^{+++}, 0.2 mg.; Zn^{++}, 0.2 mg.; Mn^{++}, 0.1 mg.; thiamine, 25 μg.; agar, 20 gm.; distilled water, 1000 ml. The medium was adjusted to a pH of approximately 6.0 and autoclaved at 15 lb. pressure for 15 min.

The cultures were grown in Petri dishes containing approximately 25 ml. of medium. In most experiments the cultures were incubated in a culture room at $25 \pm 1°$ C. Temperature experiments were carried out in small incubators and in refrigerator-incubators which varied less than 1° C. To provide uniform adequate aeration for conidial production, the lids of the Petri dishes were raised a distance of about 2 mm. by three riders made of bent strips of thin aluminum hooked over the edge of the lower half of the dish.

Difficulty was encountered in preserving viable stock cultures in test tubes in the usual way for any great time, since few or no spores were produced under these conditions. After trying various methods, it was found that the spores would withstand freezing for an indefinite time. Spores were then collected from heavily sporulating cultures and suspended in sterile distilled water in test tubes. These were immediately placed in the freezing compartment of a refrigerator where they remained until needed for inoculum. The ice was then melted slowly and a loopful of the spore suspension was used to inoculate each plate. This method provided a constant supply of viable spores at all times. Frozen spores have been so stored for 11 months and are still viable.

A wide-field stereoscopic microscope was used to count conidial heads and sporangia. The reproductive structures on the entire plate, or representative fraction thereof, were counted and recorded 3 days after inoculation.

The light conditions are described in connection with those experiments.

EXPERIMENTAL RESULTS

Under favorable laboratory conditions the fungus grows and sporulates much as it does in nature, with the exception of the many sporangia formed on artificial media. The mycelium covers a Petri dish of the above medium in approximately 54 hr. at 25° C. Conidium formation normally occurs during the second and third nights. Under most conditions the formation of sporangia lagged behind and continued longer than the production of conidia. Many small sporangia were usually present in cultures 5 days old.

Nutritional requirements for growth and sporulation. Preliminary experiments showed that this fungus is highly deficient for thiamine, and is autotrophic with respect to biotin, inositol, and pyridoxine. Without added thiamine its growth was rather sparse with few, if any, spores formed. On a medium containing 20 gm. glucose and 25 μg. thiamine per liter its growth was rapid and the aerial mycelium abundant. Conidium formation was relatively poor. Smaller amounts of sugar reduced the aerial growth and the number of conidial heads was increased. A brief summary of the essential data on this phase of the work is presented in table 1.

TABLE 1.—*The effects of concentration of glucose and thiamine upon the number of conidial heads formed*

Glucose per liter (in grams)	Thiamine per liter (in micrograms)	Number of conidial heads per plate
25	0.0	8
25	1.5	210
25	25.0	450
2	25.0	2000

These results, which show that the conidial heads are produced in greater abundance when the amount of sugar is low and the amount of thiamine is relatively high, are in full accord with the reported (1) abundance of perithecia of *Ceratostomella fimbriata* under similar conditions.

While no extensive study of the carbon requirements was made, the most abundant growth and sporulation occurred on glucose. Sucrose and lactose were relatively poor sources of carbon for growth but were fair for sporulation. Asparagine and casein hydrolysate were excellent sources of nitrogen for growth and sporulation, while nitrate nitrogen supported only sparse growth. The fungus also grew well on a number of media of natural origin, including malt extract, potato-glucose, wheat extract, V-8 juice, and autoclaved young squash fruit.

Aeration requirements for sporulation. Early in this study it was noted that few conidial heads were formed in test-tube cultures. This verified the observations of Wolf (11), which are cited above. It was further observed that more conidial heads were formed in Petri dishes with loose-fitting lids than in dishes with tight-fitting lids.

Immediately after inoculation, Petri-dish cultures were sealed with a strip of cellulose tape, so as to prevent the rapid exchange of gases. Con-

trol plates were supplied with air as described above. All plates were incubated under favorable conditions of light and temperature. The growth of the mycelium in the sealed plates was approximately equal to that in the aerated cultures. After 3 days no conidial heads were present in the sealed cultures, while 2,000 or more were present in the aerated cultures. Prolonged incubation had no effect. Numerous repetitions of this experiment gave the same results. Many small sporangia were present in all cultures 5 days old.

Thus, adequate aeration was an absolute requirement for the formation of conidia, but was not essential to vegetative growth or the formation of sporangia. Care was taken to supply the cultures with sufficient air in all other experiments. Further detailed experiments are planned to determine the actual effects of aeration, whether it provides more oxygen or removes toxic gases in the culture atmosphere.

Temperature and the formation of conidia and sporangia. Brief preliminary experiments showed that the optimum temperature for conidial

TABLE 2.—*The effect of temperature upon production of conidial heads and sporangia*

Temperature during sporulation	Conidial heads per culture	Sporangia per culture	Average diameter of sporangia
25° C.	2,000	300	60–90 μ
30° C.	150	1,300	148 μ
31° C.	0	1,200	145 μ
34° C.	0[a]	0	

[a] Abundant mycelial growth.

production was near 25° C., while excellent mycelial growth occurred as high as 34° C. No attempt was made to determine the maximum temperature which permitted growth. The numbers of conidia and sporangia produced varied greatly with the temperature changes between 25° and 34° C. In all experiments the cultures were provided with the necessary aeration and with favorable light conditions. They were exposed to continuous light at 25° C. for about 2 days to allow adequate mycelial growth to support sporulation. The cultures were then placed in incubators at the desired temperature in total darkness for about 12 to 16 hr.

The temperatures employed ranged from 18° to 34° C. Below 25° C. both growth and sporulation decreased, until few conidia were produced at 18° C. The results of several experiments are summarized in table 2. The numbers of sporangia and conidial heads represent averages of numerous cultures.

As the temperature was increased above 25° C. the number of conidial heads decreased, until the maximum temperature for their formation was reached. This appears to be approximately 30° to 31° C. As the number of conidial heads decreased with a rise in temperature, the number and size

of sporangia increased. The maximum temperature for sporangium formation was between 32° and 34° C.

The greater number and size of the sporangia at the higher temperatures is probably a reflection of the conditions unfavorable to conidium formation, rather than a direct response to temperature. It is believed that when conditions are favorable to the formation of conidia these structures are first supplied with nutrients. An exposure to light prior to sporulation in darkness was not necessary for the formation of the large sporangia at 30° C. Cultures incubated in darkness at this temperature from the time of inoculation produced many large sporangia within 3 days.

Since a change in temperature alone made a great difference in the number of conidia and sporangia formed on synthetic medium, it seemed likely that a similar effect might occur on the natural host. Pumpkin flowers and young fruits were then artificially inoculated in the garden. Two days later, when the rot was evident, the infected parts were taken into the laboratory and placed in the dark at 30° C. When observed about 14 hr. later, numerous sporangia and conidia were present on the decayed host parts. These results show that the usual absence of sporangia in nature is not due to some inhibitory substance in the host. They suggest that in nature the night temperatures at the ground level usually favor the formation of conidia, and sporangia seldom form.

Light and the formation of conidia. It was soon evident that light had little or no effect upon mycelial growth, but that the formation of conidia was governed by the light conditions. In order to determine accurately the light requirements for conidium production, it was necessary to conduct the experiments under artificial light of known constant intensity and period of exposure. For exposures to bright light the cultures were placed 15 in. below a 40-watt daylight fluorescent tube in a culture room at 25° C. A photographic exposure meter (G. E. type) gave a reading of 60 foot-candles at the level of the cultures. For total darkness the cultures were placed in a cardboard box which in turn was placed in a small darkened room at the same temperature. Some experiments were conducted in refrigerator-incubators each equipped with a 15-watt daylight fluorescent tube.

When cultures were exposed to alternate light and dark periods of approximately 12 hr. each, the average number of conidial heads produced was near 1,800 to 2,000 per culture. Conidia failed to form in cultures exposed to continuous bright light from the time of inoculation. Incubation extended beyond 3 days was without effect. Likewise, when cultures were placed in continuous total darkness conidia failed to form. It was evident that exposures to both light and darkness were necessary for the production of conidia.

Cultures placed under continuous light for 2 days to allow sufficient mycelial growth produced abundant conidial heads when subjected to a 12-hr. period of darkness. However, when the order of exposure was reversed

(2 days in darkness followed by continuous light), no conidial heads were formed. Thus, not only must this fungus have both light and darkness, but the exposures must be in a certain order (light followed by darkness) for the conidia to be produced.

Since the difference between bright light and darkness is fundamentally one of light intensity, it was thought that continuous exposure to light of low intensity might allow the formation of conidia. This was also suggested by the fact that a slight light leak in one darkness experiment permitted a few conidial heads to form. After some experimentation it was found that cultures placed under weak light (less than 1 foot-candle) for 3 days following inoculation produced an average of about 350 conidial heads per culture.

A question arose regarding the minimum length of exposures to light and to darkness required for conidium production. Some indication as to the minimum exposure to darkness was obtained by accident. During a continuous light experiment the electric power failed for a period of 2½ hr., and the cultures so exposed then formed several conidial heads.

Cultures were incubated in continuous light for 2 days after inoculation. They were then placed in total darkness for varying periods of time ranging from 1 to 10 hr., after which they were returned to light. The actual numbers of conidial heads formed varied somewhat in the several repetitions, but the results were essentially the same. The effect of the increased time of exposure to darkness on the average number (from three or more experiments) of conidial heads per culture was as follows: 1 hr., none; 2 hr., 15; 3 hr., 310; 5 hr., 1400; 10 hr., 1700.

An exposure to darkness for 1 hr. was not enough to allow the formation of conidial heads when the cultures were returned to light. A 2-hr. period of darkness is apparently near the minimum for the formation of conidia under these conditions. An increase in the dark period up to 10 hr. resulted in an increase in the number of conidial heads, but still greater periods of darkness were without effect.

It also was shown that darkness during the time of formation of the conidiophores and conidia is not necessary, and that the second exposure to light hastens the formation and maturation of conidial heads. Cultures receiving 3 hr. of darkness had not formed conidiophores at the end of this period, but conidial heads began to form about 4 hr. after being returned to the light. Duplicate cultures still in the dark at this time had not yet formed conidia. In other cultures conidial heads began to form in darkness after 10 hr.

It was likewise desirable to determine approximately the minimum exposure to light that was necessary for conidium production. Cultures were placed in darkness for 2 days and then exposed to bright light (60 foot-candles). After varying periods the cultures were returned to total darkness for 15 to 20 hr. Under these conditions an exposure of 3 hr. or less

was not sufficient to induce the formation of conidia when returned to darkness. A few conidial heads were formed in cultures receiving 4 hr. of light. As the light period was increased the number of conidial heads increased. There were approximately 200 and 1,000 produced in cultures receiving 5 and 8 hr. of light, respectively.

Light and the production of sporangia. Throughout these experiments there was no definite evidence that light played more than a minor role in the formation of sporangia. While differences in numbers were observed under different light conditions, it could not be concluded that these differences were due to light alone. Temperature was the only factor which distinctly influenced the number and size of sporangia produced.

DISCUSSION

The present investigation has shown that the production of conidia by *Choanephora cucurbitarum* is highly sensitive to relatively small changes in the environment. The important influencing environmental factors were temperature, light, and aeration. No doubt these same factors operate in nature as well as in culture. In addition, certain nutritional requirements which could not be observed in nature were revealed in culture. The fungus is deficient for thiamine which must be supplied by the culture medium. Conidium production is favored by a relatively high concentration of thiamine and by a sugar supply which allows adequate, but not excessive, mycelial growth. The effect of increased thiamine supply upon conidium formation is in agreement with the published reports of the effects of certain vitamins upon the sexual reproduction of some fungi deficient for those specific vitamins. Reports of clear examples of the influence of vitamin supply upon asexual reproduction are not so common.

The rapid growth and reproduction of this fungus made it a most satisfactory organism for experiments dealing with environmental factors. Its high degree of sensitivity, however, made it difficult to accurately duplicate all of the conditions of the experiments. These characteristics should make this fungus highly useful and desirable in classroom experiments to demonstrate the effects of the environment upon asexual reproduction.

It is unlikely that insufficient aeration is ever a limiting factor for conidium production in nature. In culture, however, it becomes a very important factor. It seems likely that this factor alone accounts for the few conidia formed in test-tube cultures. The actual beneficial effect of aeration is not clear, except that it affords rapid exchange of gases. This must act either to supply more oxygen, or to remove carbon dioxide or other toxic gases which accumulate in the culture atmosphere. Some fungi produce ammonia which must be removed by aeration (5). In case of *Choanephora cucurbitarum,* we suspect that an accumulation of carbon dioxide may be partly responsible for the inhibitory action, but only carefully conducted experiments will answer this question.

The temperature of incubation during the period of sporulation affects the numbers of both conidial heads and sporangia. The production of conidia is prevented at a temperature 2° or 3° C. lower than that which inhibits sporangium formation. Abundant mycelial growth occurs at temperatures too high to allow sporulation. It is interesting that the sporangia produced at the higher temperatures (which prevent conidium formation) are much larger than those formed at the lower temperatures. It seems logical to assume that the food materials which would have gone into the formation of conidia at the lower temperatures are, under these conditions, available for sporangium production and this abundant food supply results in a striking increase in size and number of sporangia. It was further indicated that the critical action of temperature during sporulation (in darkness), rather than the temperature during the presporulating period (light), is the factor which determines whether conidia or sporangia will form. The great increase in the number of sporangia as the temperature was increased from 25° to 30° and 31° C. is considered to be a reflection of the corresponding decrease in the number of conidial heads rather than being a direct effect of the higher temperature.

The production of sporangia on artificially infected pumpkin flowers and fruit incubated at 30° C., when only conidia were formed at 25° C., may help to explain why the sporangia have been so rarely found in nature. This temperature (30° C.) would seldom occur near the ground during the dark hours of early morning, except in the hotter regions. However, a more diligent search for sporangia after hot nights may reveal their more common occurrence in nature.

The favorable action of alternating light and darkness upon the fruiting cycle in nature is paralleled by numerous examples, such as *Pilobolus longipes* and *Coprinus* spp. reported by Buller (2). Likewise, there are numerous reports of the need for light for sporulation of various fungi in culture. The unique characteristic of *Choanephora cucurbitarum* is its failure to produce conidia both in continuous bright light and in continuous total darkness. Not only did the fungus need both light and darkness, but the exposures must occur in that order. One 2-day exposure to darkness followed by continuous light failed to induce conidium formation.

Such a situation leads one to speculate that these two conditions (light and darkness) cause entirely different effects upon the internal activity of the fungus. These may be metabolic reactions, perhaps involving two different enzyme systems. Light is apparently necessary for one reaction (A), while it inhibits the second reaction (B). If this assumption is correct, we may suggest that the substance necessary for conidium formation is synthesized in two steps, by reaction A followed by reaction B. At any rate, both reactions must occur and B must follow A. A summary of the essential results and of the proposed hypothesis is presented in figure 1.

A somewhat comparable situation seems to occur in *Diaporthe phaseo-*

lorum var. *batatatis*. This fungus formed abundant perithecia in continuous bright light but few in total darkness (10). While darkness was not essential to ascospore formation, it was decidedly beneficial.

The minimum exposure to light necessary for conidium production was approximately 4 hr. under the conditions employed. Increased exposure up to 10 or 12 hr. resulted in a greater number of conidial heads. A similar increase in the number of conidial heads was obtained when the period of darkness was increased from the minimum of 2 hr. to 8 or 10 hr. This favorable action in increasing conidium production may be a reflection of increased synthesis of the essential compound during the longer periods of exposure. Experiments also revealed that conidia were formed in continuous light of very low intensity (less than 1 foot-candle). This would indi-

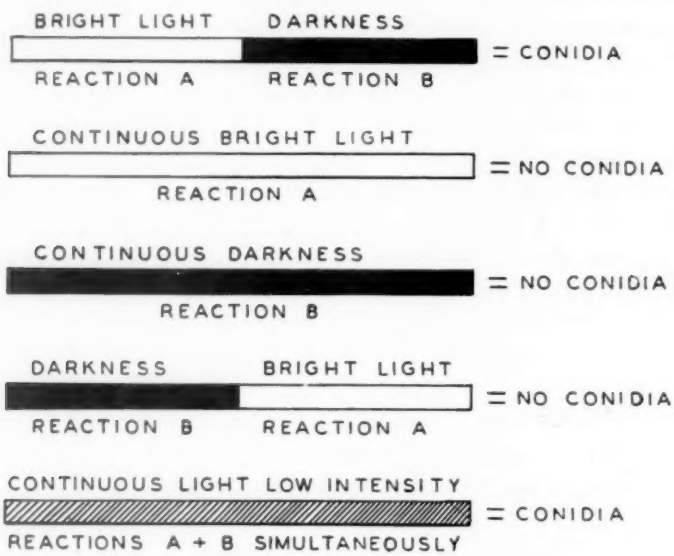

FIG. 1. Conidium formation under different light conditions, with possible necessary metabolic reactions controlled by light. Cultures were exposed to the first condition (on the left) for 2 days and to the second condition for 12 to 24 hr.

cate that both metabolic reactions took place simultaneously under these conditions. It is theoretically possible that such a range of intensity might exist.

The question arises as to whether the action of light is due to the total energy received by the fungus or to the length of exposure. It may be seen from the above data that continuous exposure for 72 hr. to light of less than 1 foot-candle resulted in the formation of approximately 350 conidial heads per culture. This number is somewhat greater than that formed in cultures receiving one 5-hr. exposure to light of 60 foot-candles. It would seem, therefore, that the length of exposure to light of a favorable intensity, as well as the total energy received by the fungus, is an important factor.

SUMMARY

The production of conidia by *Choanephora cucurbitarum* was found to be influenced by the amounts of thiamine and sugar in the medium, and by temperature, light, and aeration. Temperature was the only factor which greatly influenced the formation of sporangia. A temperature of 31° C. during sporulation was unfavorable to conidium production, but allowed the formation of many sporangia which were distinctly larger than those formed at 25° C. Aeration of the cultures was essential to the formation of conidia.

Conidia failed to form in continuous bright light and in continuous total darkness. However, conidia were produced in cultures incubated in continuous light of very low intensity. Exposure to bright light to allow adequate mycelial growth, followed by 2 hr. or more of darkness, was favorable to conidium formation. When the order was reversed (darkness followed by continuous light), no conidia were produced. The number of conidial heads was increased as the light period (following and preceding periods of darkness) was increased from 4 hr. (near the minimum) to 10 hr.

An hypothesis is proposed to explain, in part, the action of light upon conidium production by this fungus. It is based upon the belief that two metabolic reactions are involved, one favored by light and another inhibited by light.

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THE HELMINTHOSPORIA THAT ATTACK SUGAR CANE

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INTRODUCTION

Sugar cane (*Saccharum officinarum* L.) is parasitized by *Helminthosporium sacchari* (van Breda de Haan) Butler, and by *H. stenospilum* Drechsler. The former fungus causes eye-spot disease of cane (1, 8, 10, 12, 13, 14, 15, 16, 17), Napier grass (*Pennisetum purpureum* Schum.) (17, 20), and lemon grass (*Cymbopogon citratus* D.C.) (2), while *H. stenospilum* produces brown-stripe disease of cane (6, 7, 8, 12, 13, 14). With the brown-stripe disease no "runner" or streak of dead tissue extends from the primary infection towards the leaf tip as is the case with eye spot. This absence of runners has been employed by plant pathologists to separate the two parasites. It is demonstrated here that *H. sacchari* can be isolated from brown-stripe lesions and should be considered able to cause this disease as well as eye spot; inoculations to prove the point have not been performed.

A third species, *Helminthosporium rostratum* Drechsler, has been isolated from brown-stripe lesions in association with *H. sacchari*, and from eye spot of lemon grass. This rostrate species was described (5) as a doubtful parasite on dry leaves of stink grass (*Eragrostis major* L.), and has been found on dying corn leaves (*Zea mays* L.) (11).

HELMINTHOSPORIUM SACCHARI AND HELMINTHOSPORIUM STENOSPILUM

Literature Review

The author demonstrated that *Helminthosporium sacchari* was the causal agent in Hawaii of the eye spots of Napier grass and sugar cane (17). This confirmed findings by Voorhees (20) in Florida. Further, the fungus was as highly variable in Hawaii as McRae (14) and Mitra (15, 16) had found in India. McRae does not mention the outstanding difference between the conidia of *H. sacchari* and *H. stenospilum*, which is the thicker wall of the latter (6, 8). The writer briefly studied the brown-stripe disease while teaching tropical plant pathology in Hawaii. A fungus isolated in pure culture could not be distinguished from *H. sacchari* isolated from Napier grass or sugar cane diseased with eye spot (17, p. 50, footnote). Martin (13) has reported difficulty in separating the Helminthosporia cultured from eye spot and from brown stripe in Hawaii.

Materials and Methods

From September 1941 to December 1944, isolations of the species from eye spot and from brown stripe of sugar cane, and from eye spot of lemon grass, were made and studied.[1] Other isolates were received from cooperat-

[1] The writer is grateful to Dr. C. M. Tucker, Chairman of the Botany Department, University of Missouri, Columbia, Missouri, for providing facilities for pursuance of this problem (1941–42).

ing plant pathologists. *Helminthosporium stenospilum* was isolated in a single instance, from cane leaves diseased with brown stripe, sent from Cuba through the cooperation of Dr. S. C. Bruner, Estación Experimental Agronómica, Santiago de las Vegas, Cuba. The isolates and their sources are listed in table 1.

TABLE 1.—*Isolates of Helminthosporium sacchari and H. stenospilum studied; origin, host plant, and disease exhibited from which isolate was obtained. All isolates except number 53 are of H. sacchari*

Isolate number	Disease	Host plant	Variety	Locality	Isolated by
29	Eye spot	Sugar cane	.....	Clewiston, Fla.	B. A. Bourne
30	Do	do	B 3124	do	G. K. Parris
31	Do	do	do	do	do
33	Do	do	do	do	do
37	Brown stripe	do	do	do	do
40	Eye spot	Napier grass	.....	Gainesville, Fla.	C. L. Lefebvre
41[a]	Do	Sugar cane	.....	India	M. Mitra
42	Do	Lemon grass	.....	Clewiston, Fla.	B. A. Bourne
44	Do	do	.....	do	G. K. Parris
53[b]	Brown stripe	Sugar cane	.....	Cuba	do
54	Do	do	.....	Belle Glade, Fla.	C. L. Lefebvre
55	Do	do	.....	do	do
86	Eye spot	Napier grass	.....	Oahu, T.H.	G. K. Parris
88	Do	do	.....	do	do
92	Do	do	.....	do	do
93	Do	do	.....	do	do
96	Do	do	.....	do	do
101	Do	do	.....	do	do
103	Do	do	.....	Kona, T.H.	C. W. Carpenter
104	Do	do	.....	Maui, T.H.	G. K. Parris
105	Do	do	.....	Maui, T.H.	do
107	Do	do	.....	Olaa, T.H.	do
109	Do	Sugar cane	.....	Oahu, T.H.	J. P. Martin
110	Do	do	.....	do	G. K. Parris
111	Do	do	P.O.J. 2878	Kauai, T.H.	do
113	Do	do	do	do	do
116	Do	do	H 109	Maui, T.H.	do
118	Do	do	No. 5637	do	do
119	Do	do	do	do	do
120	Do	do	P.O.J. 2878	Kauai, T.H.	do
121	Do	do	do	do	do
300	Brown stripe	do	P.R. 809	Clewiston, Fla.	do
301	Do	do	do	do	do
302	Do	do	do	do	do
304	Do	do	Otaheite	do	do
305	Do	do	do	do	do
306	Do	do	B 3124	do	do
311	Do	do	Otaheite	do	do
313	Eye spot	do	do	do	do
314	Do	do	do	do	do
315	Brown stripe	do	P.R. 809	do	do
316	Do	do	do	do	do
317	Eye spot	do	do	do	do
318	Do	do	do	do	do
319	Brown stripe	do	B 3124	do	do
320	Eye spot	do	do	do	do

[a] Received subculture from J. E. Machacek, Winnipeg, Manitoba, Canada, who obtained culture from Centraalbureau voor Schimmelcultures, Baarn, Holland.

[b] Isolated from material sent by S. C. Bruner, Estación Experimental Agronómica, Santiago de las Vegas, Cuba.

Small portions from diseased leaves which showed the desired symptom were excised with a flamed razor blade. These pieces were dipped momentarily in 95 per cent alcohol and then submerged for 3 to 5 min. in calcium hypochlorite solution in water (approximately 0.35 per cent). They were then plated on agar without washing. Pure cultures were obtained in this way. Media were cornmeal, 1 per cent sucrose, and standard nutrient agars, as used previously (17). Cultures were kept in the dark in an oven at 27° to 28° C. The data on spore size and septation of conidia in table 2 were obtained by measuring 150 spores per isolate. Relative speeds of growth

TABLE 2.—*Length, width, and septation of conidia of Helminthosporium sacchari and H. stenospilum, produced on cornmeal agar, at 27°–28° C.*

Isolate number	Length in μ		Width in μ		Septa	
	Range	Mean	Range	Mean	Range	Mean
			H. sacchari			
29	36–74	47	8–16	11	3–6	5
37	36–104	64	10–16	12	3–9	6
40	42–105	62	8–14	10	4–8	6
41	30–96	57	10–16	10	3–10	6
42	25–65	52	8–14	10	3–5	4
54	24–80	45	8–16	10	2–7	4
55	36–96	71	10–16	14	3–9	6
86, 88, 92, 93, 96, 101, 103, 104, 105, 107	25–113[a]	69	8–17	11	3–11	6
109, 110	27–76	55	9–16	11	2–9	6
111	24–105	60	8–12	10	3–8	6
113	30–118	68	9–15	11	4–9	6
118	30–112	65	8–12	11	4–10	7
119	30–104	65	8–16	12	4–10	6
120	24–96	61	6–14	11	3–8	6
121	30–116	70	8–12	11	2–8	6
300	32–93	64	8–16	13	3–9	6
301	36–128	84	10–16	13	5–11	7
302	32–92	61	10–16	14	3–9	6
304	36–92	61	9–18	14	2–8	6
305	32–96	62	10–18	14	3–9	6
306	36–92	63	10–18	13	3–9	6
307	36–132	71	10–16	12	2–9	6
309	44–112	72	12–20	16	3–10	9
311	40–112	60	10–16	12	3–10	6
312	32–92	57	10–16	13	2–8	5
313	40–124	86	12–16	14	3–11	7
314	32–84	61	10–16	13	3–7	5
316	32–104	65	8–16	13	3–9	6
			H. stenospilum			
53	52–133	85	10–16	14	5–11	7

[a] Obtained from Parris (17).

TABLE 3.—*Type of growth, quantity of growth, and spore formation by isolates of Helminthosporium sacchari and H. stenospilum, at 28° C., on various media*

Isolate number	1 per cent sucrose agar		Amount of mycelial growth on cornmeal agar (diam., 7 days)	Spore formation on standard nutrient agar after 23 days
	Type of mycelial growth	Amount of mycelial growth (diam., 7 days)		
		Helminthosporium sacchari		
37	Not dichotomous	51 mm.	38–40 mm.	.....
38	Do	82 mm.	68–70 mm.	.....
39	Do	70–72 mm.	42–46 mm.	.....
54	Do	83 mm.	75 mm.	None
55	Dichotomous	44–46 mm.	60 mm.	None
96	.....	.....	.....	None
103	.....	.....	.....	Spores
104	.....	.....	.....	None
107	.....	.....	.....	None
109	.....	.....	.....	Spores
110	.....	.....	.....	Spores
111	Not dichotomous	.....	.....	None
113	Dichotomous	.....	.....	Spores
115	Do	.....	.....	.....
116	Not dichotomous	.....	.....	None
117	.....	.....	.....	None
118	Not dichotomous	.....	.....	None
119	Dichotomous	.....	.....	None
120	Do	.....	.....	None
121	Not dichotomous	.....	.....	None
300	Dichotomous	28–32 mm.	55 mm.	None
301	Do	35–53 mm.	43–50 mm.	.....
302	Do	36–42 mm.	47–50 mm.	.....
304	Do	20–35 mm.	42–55 mm.	.....
305	Do	43–47 mm.	43–52 mm.	.....
306	Do	31–35 mm.	47–54 mm.	.....
307	Do	31–33 mm.	53–57 mm.	.....
311	Do	17–26 mm.	48–51 mm.	.....
312	Do	16–18 mm.	68–70 mm.	.....
313	Do	38–47 mm.	42–53 mm.	None
314	Do	20–30 mm.	58–60 mm.	.....
315	Do	50–60 mm.	59–62 mm.	None
316	Do	34–37 mm.	35–42 mm.	.....
319	.....	.....	.....	None
320	.....	.....	.....	None
		Helminthosporium stenospilum		
53	Not dichotomous	82 mm.	70 mm.	Spores[a]

[a] Spores formed in less than one week.

by different isolates on the same medium, or types of growth on different media (Table 3), were gathered from plates seeded simultaneously with small squares of young hyphae plus agar, ½ cm. square. The pH of all media was approximately at neutrality. Experiments were repeated until uniform observations were obtained. Nuclear studies were possible using the acid fuchsin stain technique (18). Drawings (Fig. 1 to 6) were made with the aid of a camera lucida.

Helminthosporium Sacchari Isolated from Brown-Stripe Lesions

Two cultures (Table 1, No. 54, No. 55) isolated from Florida sugar cane

diseased with brown stripe, had the same thin-walled conidia (Fig. 2, No. 54, No. 55) as the *Helminthosporium* cultured from eye spot of Napier grass in Hawaii, from eye spots of cane and of lemon grass sent from Florida, and from brown stripe of cane in Hawaii. Cane leaves, varieties Otaheite,

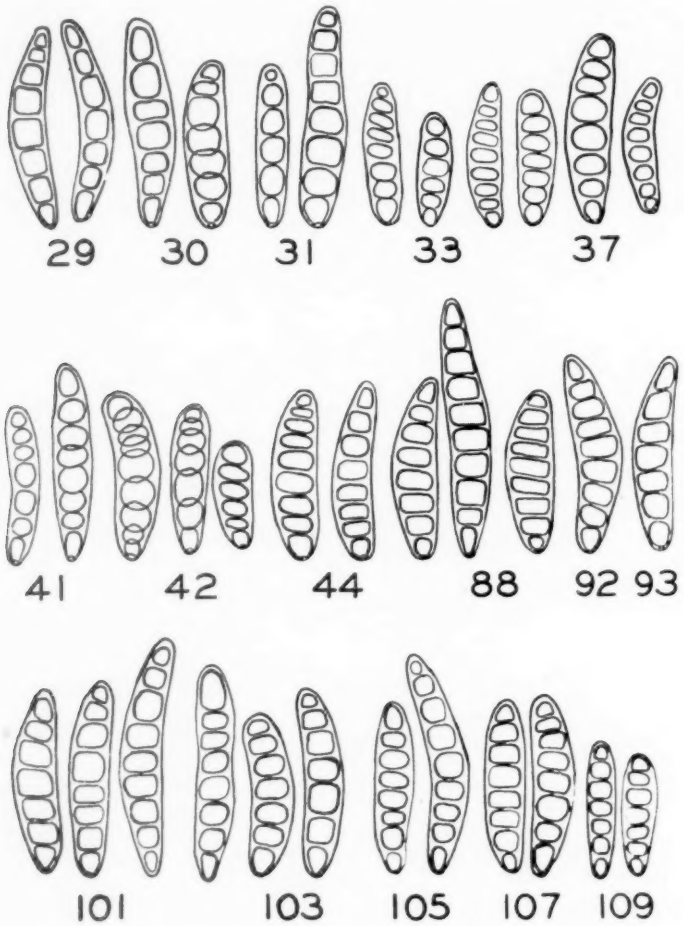

FIG. 1. *Helminthosporium sacchari.* Conidia of 16 isolates produced on cornmeal agar at 27°–28° C. (×300). Isolates 29, 30, 31, 33, 41, and 109 from eye spot on sugar cane; isolates 88, 92, 93, 101, 103, 105, and 107 from eye spot on Napier grass; isolate 37 from brown stripe on sugar cane; and isolates 42 and 44 from eye spot on lemon grass.

B 3124, and P.R. 809, showing brown stripe, were received from Florida. An *Helminthosporium* was successfully isolated (Table 1, isolates 300–302, 304–306, 311). Other diseased leaves, which lacked the typical "runner" phase but which had an eye spot as well as brown stripe, also were received from Florida; an *Helminthosporium* was cultured from both types of lesions (Table 1, isolates 313–320). In all instances the fungus isolated was *H. sacchari.* Apparently *H. sacchari* can cause a brown-stripe disease of sugar

cane as well as eye spot, while *H. stenospilum* so far as is known produces only brown stripe. In figure 3, C are shown the thick-walled conidia of isolate 53 (*H. stenospilum*), and in figure 3, D the relative widths of *H. stenospilum* and of *H. sacchari* are compared.

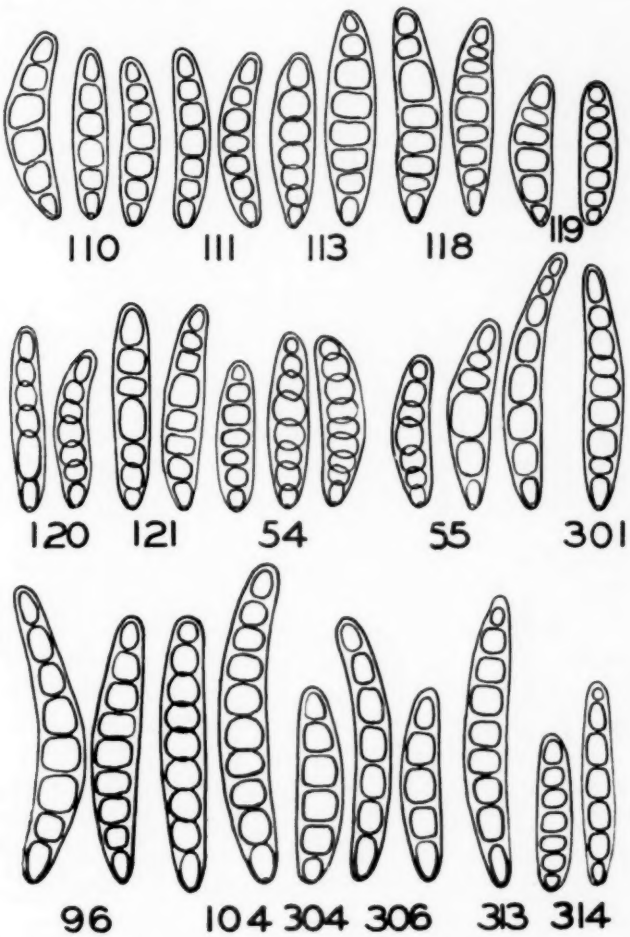

FIG. 2. *Helminthosporium sacchari.* Conidia of 16 additional isolates produced on cornmeal agar at 27°–28° C. (×300). Isolates 110, 111, 113, 118, 119, 120, 121, 313, and 314 from eye spot on sugar cane; isolates 54, 55, 301, 304, and 306 from brown stripe on sugar cane; isolates 96 and 104 from eye spot on Napier grass.

Variability of spore dimensions is great in *Helminthosporium sacchari,* as others have found. The mean length of the conidium produced on cornmeal agar at 28° C. varied in these studies from 45 μ to 86 μ, with mean septation ranging from 3 to 8; mean widths ranged from 10 μ to 16 μ (Table 2 and Fig. 1 to 3). The conidia of the single isolate of *H. stenospilum* measured 52–133 μ (mean 85 μ) ×10–16 μ (mean 14 μ), with 5–11 (mean 7) septa; these dimensions agree with other reports for this species (6, 8).

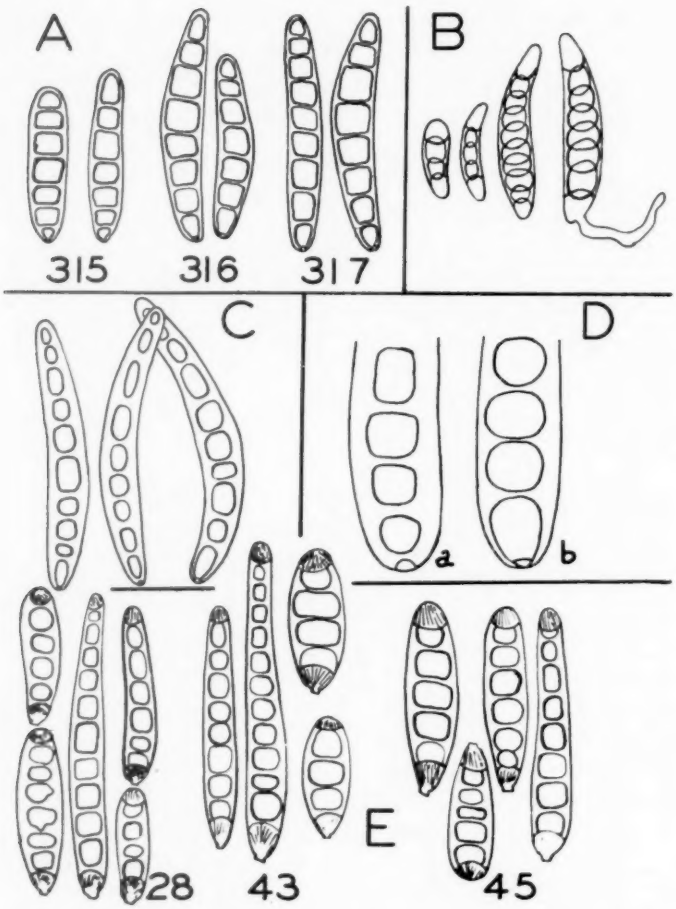

FIG. 3. A. *Helminthosporium sacchari.* Conidia of 3 additional isolates, produced on cornmeal agar, at 27°–28° C. (×300). Isolates 315, 316 from brown stripe on cane, variety P.R. 809; isolate 317 from eye spot on same variety of cane. B. Conidia of the brown stripe of cane fungus in Hawaii, said to be of *H. stenospilum* but believed to be of *H. sacchari* (×300). Redrawn from Martin (13). C. *H. stenospilum.* Conidia produced on cornmeal agar, at 27°–28° C. (×300). Note thick wall. D. Conidial walls of (a) *H. stenospilum* and (b) *H. sacchari,* to illustrate relative widths (×700). E. *H. rostratum.* Conidia of 3 isolates, produced on cornmeal agar, at 27°–28° C. Isolate 28 from corn, isolate 43 from lemon grass, and isolate 45 from sugar cane (×300).

Growth on Artificial Media

McRae (14) found it was difficult to separate *Helminthosporium sacchari* from what he considered to be *H. stenospilum,* but which probably was *H. sacchari,* by growth on artificial media. Faris (8) believed that he could distinguish *H. stenospilum* from *H. sacchari* by growth at 25° C. on 1 per cent sucrose agar, but Bourne (1) showed that the dichotomous type of growth, observed by Faris for *H. sacchari* at 28° C., was not formed at 23.5° C. Parris (17) found, however, that *H. sacchari* produced dichoto-

mous growth at 21° C., but not at 16° C. on sucrose agar. Faris (8) reported that *H. stenospilum* formed broader concentric rings on artificial media than *H. sacchari* under similar conditions.

The writer grew a number of isolates of *Helminthosporium sacchari* and the single isolate of *H. stenospilum,* on 1 per cent sucrose at 28° C.; growth of the latter agreed with that described by Faris (8), but similar growth can be produced by some isolates of *H. sacchari* which need not always grow in dichotomous fashion (Table 3). It does not seem to be possible to separate the two species on sucrose agar. On cornmeal agar at 28° C. (Table 3) there is less variation between the radial growth of different isolates of *H. sacchari* than occurs on sucrose agar, and the growths of *H. sacchari* and *H. stenospilum* are very similar; it seems impossible to separate the two species on this agar. Three isolates of *H. sacchari,* one from Hawaiian plant material, formed spores at 28° C. after 23 days on standard nutrient. In contrast, *H. stenospilum* formed conidia in less than a week on this agar.

The pathogenicity of the isolates of *Helminthosporium sacchari* from Hawaii is known to have been vigorous once, but none of the others, or of *H. stenospilum,* has been tested for this property.

Nuclear Studies

Little is known of nuclear conditions in most of the graminicolous Helminthosporia. Dickinson (4), who studied *Helminthosporium pedicellatum* Henry, *H. monoceras* Drechsler, and an unidentified Brachysporium type of Helminthosporium, found that vegetative mycelium was multinucleate. All hyphae with slightly swollen ends, which appeared to be young conidiophores, had a uninucleate apical cell in the early stages. In somewhat later stages, the apical cell contained two nuclei; sometimes these two nuclei were the products of a single nuclear division. Shands and Dickson (19) reported that germinating conidia, germ tubes, and mycelial cells of *H. gramineum* Rabenhorst were multinucleate. This was confirmed by Graham (9). Christensen and Davies (3), studying *H. sativum* P., K., and B. and *H. tetramera* McKinney, found that the number of nuclei in hyphal cells varied from one to six. Young conidiophores often contained a single nucleus.

In *Helminthosporium sacchari,* the number of nuclei in vegetative hyphal cells varies from one to five, with two the number most commonly encountered (Fig. 4, Ba–Bd). The number of nuclei per cell close to the germinating spore is greater (Fig. 4, Bc) than at some distance (Fig. 4, Ba–Bb). Intermediate cells of a germinating conidium contain two to four nuclei (Fig. 4, Ac). The young conidiophore contains a single nucleus (Fig. 4, Ca–Cl, Da–Di), but soon this number is doubled (Fig. 4, Ea–Eb), and as soon as conidial formation begins, further division takes place (Fig. 4, Ec–Ed). In the young and still nonseptate conidium, two nuclei have been seen and the cells of the conidiophore are now binucleate (Fig. 4, Ef).

In *Helminthosporium stenospilum* the number of nuclei in the vegetative cells varies from one to four, with two the most usual number (Fig. 5, Ba–Bf). Occasionally, nuclear division can be seen taking place (Fig. 5, Be). Within the germinating conidium the intermediate cells reveal one to two nuclei, though further studies may show that this number is con-

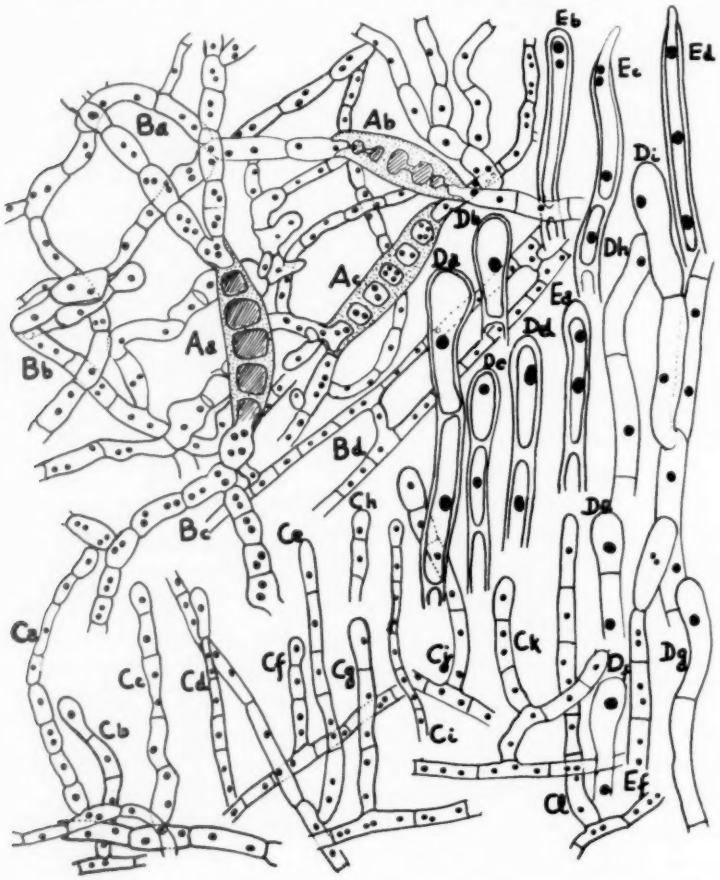

FIG. 4. *Helminthosporium sacchari.* A. Germinating spores (×300). B. Vegetative hyphae (×300). C. Young conidiophores containing a single nucleus in terminal cells (×300). D. Young conidiophores containing a single nucleus in terminal cells (×600). E. Ea–Ed, conidiophores with binuclear terminal cells (×600); Ef, formation of conidium from conidiophore (×300).

servative. The young conidiophore of *H. stenospilum* is binucleate (Fig. 5, Ca–Co). Cells immediately behind the young swollen conidiophore are usually also binucleate, but occasionally a uninucleate cell is seen (Fig. 5, Ch). In a single instance the young conidium was found to contain a single nucleus (Fig. 5, Db).

HELMINTHOSPORIUM ROSTRATUM

While culturing from brown-stripe lesions on the Otaheite variety of

sugar cane, and from eye-spot lesions on lemon grass sent from Clewiston, Florida, the fungus *Helminthosporium rostratum* Drechsler (5) infrequently was obtained. Tissue platings yielded this fungus in pure culture from lemon grass in two instances, and in mixed culture with *H. sacchari* in a single instance from sugar cane.

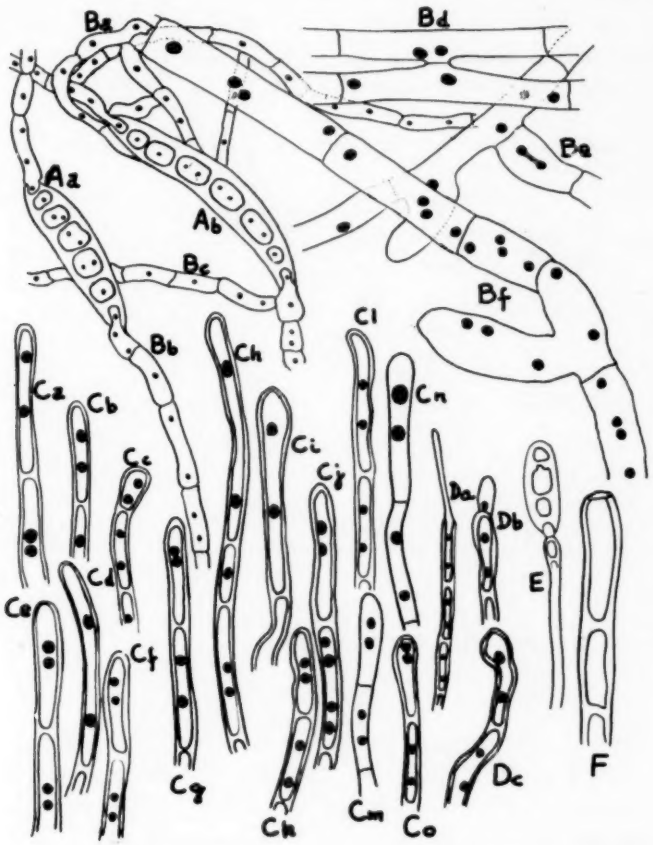

FIG. 5. *Helminthosporium stenospilum*. A. Germinating spores (×300). B. Vegetative hyphae; Ba–Be, ×300; Bd–Bf, ×900. In Be, note nuclear division taking place. C. Young conidiophores containing two nuclei in terminal cell (×600). D. Possible conidial formation (×600). E. Young conidium attached to conidiophore (×300). F. Old conidiophore showing scar from which conidia have been dislodged (×600).

Spore Dimensions

One hundred and fifty spores were measured from each of four isolates of *Helminthosporium rostratum* and results are given in table 4 below. Rostrate and non-rostrate spores were measured separately; Drechsler (5) combined their measurements. The dimensions of the spores of the fungus from corn, lemon grass, and sugar cane are close enough to cause no doubt that they are the isolates of the same species.

Nuclear Studies

Germinating spores stained with acid fuchsin (18) showed that two nuclei per cell seem most common in vegetative cells (Fig. 6, Ba–Bc), with a single nucleus present in the swollen terminal cell of the young conidiophore (Fig. 6, Ca–Cc; Da–De). Even though enlargement of the terminal cell (Fig. 6, Ea) takes place, or a cell wall appears (Fig. 6, Eb, Ed–Ee), the solitary nuclear condition of the conidiophore persists. In later stages, the terminal cell is binucleate. A protoplasmic connection from cell to cell of the conidiophore was observed (Fig. 6, Ea, Eb, Ee).

TABLE 4.—*Spore dimensions of 4 isolates of Helminthosporium rostratum, compared with findings by Drechsler (5)*

Host plant	Associated symptom	Type of spore	Length in μ		Width in μ		Septa	
			Range	Mean	Range	Mean	Range	Mean
Stink grass[a]	Dried leaves	Rostrate and non-rostrate	32–184	.....	14–22	.....	.....	.....
		Rostrate	.....	.....	.....	.....	8–15	.....
		Non-rostrate	.....	.....	.....	.....	3–9	.....
Corn[b]	Dried leaves	Rostrate	64–136	87	9–16	12	6–10	7
		Non-rostrate	16–72	44	9–16	12	1–8	5
Lemon grass	Eye spot	Rostrate	56–160	78	10–16	14	7–15	9
		Non-rostrate	24–72	52	10–16	12	2–10	7
Lemon grass	Eye spot	Rostrate	68–108	84	12–15	13	8–11	9
		Non-rostrate	24–76	60	10–14	12	2–10	7
Sugar cane	Brown stripe	Rostrate	60–140	82	12–18	15	7–12	8
		Non-rostrate	24–74	52	10–15	12	2–10	6

[a] After Drechsler (5).
[b] Culture received from C. L. Lefebvre.

DISCUSSION

From these studies emerges the fact that not all brown-stripe lesions on sugar cane are produced by *Helminthosporium stenospilum*. *H. sacchari* can be the pathogen in many instances.

From Martin's (13, p. 66) statement, "Measurements . . . demonstrated that it was difficult to distinguish brown-stripe spores from eye-spot spores," and from the writer's corroboration of this (17), it is concluded that the thicker-walled *Helminthosporium stenospilum* is rare in Hawaii, if present at all. If this species does not occur there, what of the ascigerous stage found in 1931 by C. W. Carpenter (12), in a culture of an *Helminthosporium* isolated from brown stripe? Carpenter's drawings (13) show a helicoid arrangement of the spores in the ascus, which places the fungus in the genus *Cochliobolus* (7). However, the conidia pictured (Fig. 3, B) identified as *H. stenospilum*, which they obtained in pure culture. The com-*Cochliobolus stenospilus* for the perfect stage of an *Helminthosporium* Martin (13, p. 64), Matsumoto and Yamamoto in 1935 proposed the name are thin-walled and therefore thought to be of *H. sacchari*. According to

bination *Cochliobolus stenospilus* (Drechs.) M. and Y. is used (13) for the sexual stage of the brown-stripe pathogen. No formal description has been released by the Japanese so the combination is not a valid one. Since *H. sacchari* can cause a brown stripe of cane similar to the disease described by Drechsler (6) and by Faris (8), and to the best of the writer's knowledge *H. stenospilum* has not been cultured in Hawaii, then the *Cochliobolus* found by Carpenter could be the sexual state of *H. sacchari*.

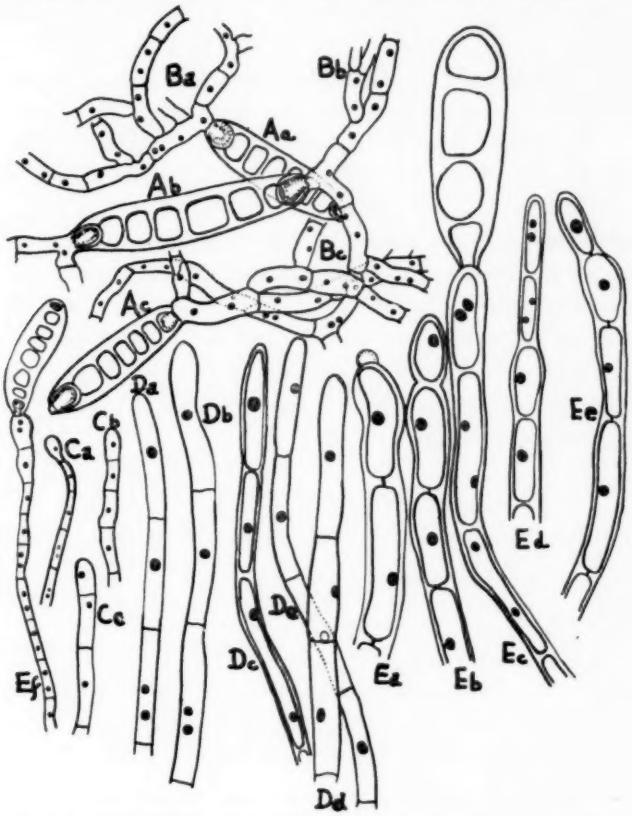

FIG. 6. *Helminthosporium rostratum*. A. Germinating spores (×300). B. Vegetative hyphae (×300). C. Young conidiophores with single nucleus in terminal cells (×300). D. Young conidiophores with single nucleus in terminal cells (×600). E. Stages in conidial formation (×600, except Ef which is ×300). Note protoplasmic connections from cell to cell (Ea, Eb, Ee).

The writer was unable to induce the large number of isolates of *Helminthosporium sacchari* in his possession, and the single isolate of *H. stenospilum*, to form a sexual stage in culture.

SUMMARY

Isolates from eye spots of Napier grass, sugar cane, and lemon grass, and from brown stripe of cane in Florida and Hawaii, have yielded *Helminthosporium sacchari* (van Breda de Haan) Butler. This species has

not previously been associated with the brown-stripe disease, which was described as due to the congeneric species *H. stenospilum* Drechsler. Apparently the thinner-walled *H. sacchari* can produce eye spot as well as brown stripe.

Conidial dimensions and septation are unreliable to separate *Helminthosporium sacchari* and *H. stenospilum,* nor can growth on 1 per cent sucrose or cornmeal agars at 27°–28° C. be used. The outstanding difference between the two species is the thicker wall of the conidium of *H. stenospilum.*

The young conidiophore of *Helminthosporium sacchari* contains but a single nucleus, while the homologous structure in *H. stenospilum* contains two nuclei. Vegetative hyphae of both species contain one, two, or sometimes as high as six nuclei, but with two nuclei per cell the most common number.

Helminthosporium rostratum Drechsler can be isolated from brown-stripe lesions of sugar cane and from eye spot of lemon grass, in the former instance in company with *H. sacchari.* This species has been considered to be a saprophyte. A single nucleus is present in the young conidiophore.

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SPORULATION OF COLLETOTRICHUM LINDEMUTHIANUM IN CULTURE[1]

R. S. Mathur, H. L. Barnett, and Virgil Greene Lilly

(Accepted for publication August 30, 1949)

The sporulation of *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav., cause of anthracnose of beans, is reported to be sparse and erratic on many natural and synthetic media. Coons (5) reported acervulus formation in Difco dehydrated and home-made media. Schaffnit and Böning (19) used Crone's solution as the basal medium and found that their cultures sporulated when organic sources of nitrogen, especially peptone, were used. Hopkins (8) reported sporulation of the *beta* strain of the fungus on potato-dextrose agar acidified with lactic acid. However, none of these media is reported to be as satisfactory for obtaining good sporulation as autoclaved bean pods and Lima-bean agar.

It is difficult to determine accurately the factors in bean plugs and Lima-bean agar which favor sporulation. Hence, our first attempts in this investigation were to devise a synthetic medium which would support good sporulation, and to obtain further information regarding the effects of various nutritional and environmental factors upon the production of conidia by this fungus. While this approach was used with some success, it proved advantageous in the end to study the problem in another way. By treating a natural medium with Norit the factors which stimulated sporulation were largely removed. Various substances were added to this purified basal medium and the effect on sporulation was then studied.

MATERIALS AND METHODS

An isolate of the *gamma* strain of *Colletotrichum lindemuthianum* was obtained from Cornell University. A single-spore culture derived from it was used throughout this investigation. After preliminary work, a glucose-neopeptone medium was found to support better sporulation than any other medium studied. It contained 2.8 gm. glucose, 1.23 gm. $MgSO_4 \cdot 7\ H_2O$, 2.72 gm. KH_2PO_4, 2.0 gm. neopeptone (Difco), 20 gm. agar, and double-distilled water to make 1000 ml.

However, when this medium was boiled with 5 gm. of Norit (activated charcoal) per liter for a few minutes and the fungus was grown on the treated medium, there was almost no sporulation. The Norit-treated medium was, therefore, useful in studying the effects of vitamins and other substances upon the sporulation of this fungus.

The possible vitamin deficiencies of the *gamma* strain were tested by

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growing it in 25 ml. of glucose-casein hydrolysate medium (13) in 250-ml. Erlenmeyer flasks at 25° C. Thiamin and pyridoxine were added at the rate of 100 μg. per liter, while biotin and inositol were added at the rates of 5 μg. and 5 mg. per liter of the medium, respectively.

The inoculum was prepared by growing the fungus in the glucose-casein hydrolysate medium for 2 to 3 weeks. Contents of a flask then were transferred and blended for 30 sec. in a Waring Blendor (18), and one drop of the suspension of cut mycelium was used in inoculating each flask or Petri dish. In a majority of experiments on sporulation, the Petri dishes were incubated at 25° C. In one experiment, however, where the effects of vitamins and temperature were studied, the plates were incubated at 15°, 20°, 25°, 30°, and 35° C. One set of plates was incubated at alternating temperatures of 20° and 30° C. For the first 48 hr. the plates were kept at 20° C.; they were then removed to an incubator kept at 30° C. for 48 hr. This schedule of alternating temperatures was maintained until the end of the experiment. After 10 days, the diameters of colonies were measured, and spore harvests were taken by scooping out four colonies of each treatment and blending them for 30 sec. in a Waring Blendor. The volume was made up to 25 ml. per colony and the contents labeled and stored at 0° C. Spores were counted by use of a haemocytometer. From each treatment 10 drops were sampled at random and from each drop spores were counted in five different fields of the microscope. Total numbers of spores per colony were calculated. Further details are described in connection with the individual experiments.

EXPERIMENTAL WORK

Especially important among the factors which influence sporulation are the kind and amounts of nutrients contained in the substrata, and the physical environment. The nutritional factors intensively investigated were the sources of carbon, nitrogen, and vitamins. Of the physical factors studied, temperature was the most important. The results of numerous preliminary experiments were reported below.

The following plant tissues were sterilized by autoclaving and tested for suitability as substrata for sporulation of this fungus: bean pods, stems of bean, wheat, barley, alfalfa, rose, red clover, and white sweetclover. Within a week the fungus sporulated abundantly on bean pods and the stems of bean and wheat; moderate sporulation was observed on white sweetclover and barley. Alfalfa, red clover, and rose did not favor sporulation.

Several infusion agars (17) were tried in these concentrations (2×, 1×, and 0.5×): Malt extract, potato-dextrose, oatmeal, whole wheat, Lima-bean, and pea. While little or no sporulation occurred on malt, potato-dextrose, oatmeal, whole wheat, and cornmeal agars, moderate sporulation was observed on Lima-bean and pea agars with 1× and 2× concentrations, and sporulation was excellent on these two agars at 0.5× concentration.

As a result of the experiments with natural media it was concluded that

the concentration as well as the constituents of the media used were important factors in the sporulation of this fungus. These conclusions were confirmed when synthetic media were tested; however, sporulation was poor under these conditions. The effect of varying concentrations of sucrose and neopeptone were next tested. Five concentrations of sucrose (60, 30, 15, 7.5, and 1.875 gm. per liter) and four concentrations of neopeptone (10, 5, 2.5, and 0.625 gm. per liter), 20 combinations in all, were tried. Good sporulation was observed with 0.1875 gm. sucrose and 2.5 gm. neopeptone. As a result of further tests the medium described in detail in the previous section of this paper was adopted for further study. Sporulation on this medium (glucose-neopeptone) was as abundant as on autoclaved bean pods.

These results are in accordance with the experience of Klebs (10), Coons (4), and Leonian (12), who found that a change of constituents and a lowering of the concentration of food favored spore production in many fungi.

KINDS OF INOCULUM

Preliminary experiments showed that sporulation was significantly better when spores were used as a source of inoculum than when mycelium was used. An experiment was carried out with Norit-treated glucose-neopeptone medium to confirm this observation. Four kinds of inoculum were used: blended mycelium, disks of mycelium on agar, spores washed free of matrix, and spores with matrix. All of the cultures were incubated at 20° C. for 11 days. No conidia formed on plates inoculated with blended mycelium. Sporulation was poor on plates inoculated with mycelial disks or with spores washed free of matrix. Moderate sporulation was observed in plates inoculated with spores with matrix. The experiment was repeated thrice, with similar results.

VITAMIN STUDIES

Vitamin deficiencies. Sporulation (sexual and asexual) of vitamin-deficient fungi has been found to depend on an adequate supply of the vitamins in question (1, 14). It appeared desirable, therefore, to test this fungus for possible deficiencies for water-soluble vitamins, and to further investigate the influence of such vitamins on asexual reproduction. The fungus was grown at 25° C. in 250-ml. Erlenmeyer flasks in 25 ml. of the casein hydrolysate medium to which known quantities of thiamin, pyridoxine, inositol, biotin, and a combination of all four vitamins were added. One series of flasks, used as a control, did not contain vitamins. Two flasks from each treatment were harvested every 4–5 days. The results of this experiment indicate that appreciably better growth occurred in the series containing inositol and biotin than in the control flasks. This was true only during the early stages of growth. By the eighteenth day of incubation, the weight of the control cultures was equal to the weight of those receiving inositol or biotin. Pyridoxine alone was essentially without effect on either the rate or amount of growth. It appears, therefore, that the *gamma* strain

is partially deficient for inositol and biotin. These conclusions were confirmed in an extensive experiment in which thiamin, inositol, and biotin were used in all possible combinations. These cultures were incubated at 15°, 20°, 25°, and 30° C. Since this paper reports the factors found to influence sporulation, rather than growth, only the essential facts established by this experiment will be given. Growth was poor at 30° C. irrespective of the vitamins added to the medium. The rate of growth at 15° C. was less than at 20° and 25° C., but there was no significant difference in maximum weight of mycelium attained at these three temperatures after 24 days' incubation. Thiamin alone depressed growth at these temperatures, but addition of either inositol or biotin or both to thiamin overcame the depression caused by thiamin. In fact, these three vitamins together stimulated early growth

TABLE 1.—*The effect of thiamin, inositol, and biotin on sporulation of Colletotrichum lindemuthianum growth on glucose-neopeptone Norit-treated medium. Cultures incubated at various temperatures*

Treatment	Approximate number of spores per colony (in millions)			
	15° C.	20° C.	25° C.	20–30° C.
Norit-treated glucose-neopeptone medium	72	68	59	12.0
Thiamin	928	532	466	0.38
Inositol	465	1272	662	1.3
Biotin	385	1143	576	2.8
Thiamin, inositol, biotin	624	1253	441	504.0
Untreated glucose-neopeptone medium	979	1830	565	270.0

to a greater degree than any other combination of vitamins tested. Thiamin is thus a growth depressant or a growth stimulant for this fungus, depending upon whether other vitamins are absent or present in the medium. The effect of vitamins on growth of the *gamma* and other strains of this fungus are to be reported elsewhere (15).

Effect of vitamins on sporulation at different temperatures. Considering that the vitamins caused variations in growth at different temperatures, it was thought that, under the same conditions, differences in sporulation might be observed. An experiment was carried out in which the different vitamins in known quantities were added, singly or in combination, to the Norit-treated glucose-neopeptone medium. The inoculated Petri dishes were incubated at 15°, 20°, 25°, 30°, and 35° C. In addition, one set of plates was incubated at 20° and 30° C. alternating—two days at one temperature and then transferred to the other temperature, repeated until the end of the experiment. Spore counts were made after 10 days.

The relevant data summarized in table 1 show that the vitamins, singly or in combination, replace to a greater or lesser degree the substances removed from neopeptone by treating it with activated charcoal. In general, more sporulation occurred at 20° C. than at any other temperature used.

If we assume that differences in amount of sporulation must be greater than twofold between treatments to be significant, there was no essential difference between the sporulation at 15° and at 25° C. Sporulation was very sparse at 30° and absent at 35° C. Cultures incubated at 20°–30° C. sporulated better than cultures incubated continuously at 30° C.

The colony diameters were measured at the time of harvest and did not vary greatly with the vitamin treatments, but temperature did affect radial growth. Colonies grown at 20° C. had approximately twice the diameter of colonies at 15° C. Colonies grown at 25° had slightly greater diameter than those cultured at 20° C. Radial growth at alternating temperatures was less than at 15° C. There seemed to be little or no correlation between the amount of radial growth and sporulation.

Effect of vitamins, matrix, and other substances on sporulation. As noted earlier, the *gamma* strain of *Colletotrichum lindemuthianum* was partially deficient for inositol and biotin. It was also noted that when the glucose-neopeptone medium was boiled with Norit, the ability of the medium to support good sporulation was almost completely lost (Table 1). This loss apparently was due to the adsorption by Norit of vitamins or other substances present in neopeptone. Spores with matrix, used as a source of

TABLE 2.—*The effect of vitamins and other substances on sporulation of vitamin-starved mycelium of Colletotrichum lindemuthianum at 20° C.*

Treatment[a]	Number of disks treated	Average number of acervuli per disk[b]
Inositol	10	4
Thiamin	9	2
Biotin	9	4
Thiamin, inositol, and biotin	4	12
Extract of spores and matrix	23	6
Autoclaved filtrate of spores, matrix, and mycelium	14	23
Eluate recovered from Norit used to treat neopeptone	3	19
Distilled water	47	0

[a] The vitamin solutions in which the disks were soaked had the following concentrations: thiamin, 20 μg/ml., inositol, 1 mg./ml., and biotin, 1μg/ml.

[b] Disks soaked in pea and bean extracts also produced several acervuli each. However, these extracts contained considerable food as well as vitamins.

inoculum, stimulated greater sporulation than did spores washed free of matrix. Pea and bean agars were favorable for abundant sporulation. From these observations it was thought that the sporulation-stimulating factors, lost from glucose-neopeptone medium during treatment with Norit, might be replaced by certain known and unknown substances. This hypothesis was tested in the following way.

Circular disks, 7 mm. in diameter, were cut aseptically from nonsporulating mycelium grown on Norit-treated glucose-neopeptone medium for 2 to 3 weeks. The disks were soaked in distilled water as control and also in

water solutions of substances under study, for 24 hr. at 20° C. Later they were planted on 2 per cent pyridine-purified water agar in Petri dishes and incubated at 20° C. Within a week spore-bearing dark acervuli appeared on treated disks, while none appeared on disks soaked in distilled water. The data in table 2 indicate that sporulation was most abundant on the disks soaked in the filtrate of spores, mycelium, and matrix, eluate from Norit, and with bean and pea extracts. Treatments with the vitamins and spore matrix also resulted in significant numbers of acervuli. In view of the failure of acervuli to form on starved mycelium in the control, it seems clear that vitamins, and possibly other substances, are required for sporulation. The absence of food in this experiment, which would limit synthesis of vitamins by the mycelium, made this test rigorous and conclusive.

CARBON SOURCES

Hawker (6) reported that the specific chemical structure of carbon sources influenced sporulation of *Melanospora destruens*. The following experiment was designed to study how different sources of carbon affected

TABLE 3.—*The effect of different carbon sources on growth and sporulation of Colletotrichum lindemuthianum when grown on glucose-neopeptone medium for 10 days at 25° C. All carbon sources were used at a rate which supplied 1.1 gm. carbon per liter*

Carbon source	Diameter of colony (mm.)	Number of spores per colony (in millions)
D-xylose	44.0	612
D-glucose	44.38	592
Sucrose	47.6	497
D-fructose	39.37	382
D-galactose	33.3	381
Maltose	50.0	321
D-mannose	32.5	174
Lactose	34.87	98
Mannitol	39.5	66
L-sorbose	12.1	36
Soluble starch	40.3	27
Sorbitol	27.33	7
None	40.37[a]	3

[a] The growth under this treatment was sparse. The amount of mycelium formed was considerably less than with other treatments.

the sporulation of *Colletotrichum lindemuthianum*. The glucose (1.1 gm. carbon per liter) in the glucose-neopeptone medium was replaced with equivalent quantities of other carbon sources. All cultures were incubated at 25° C.; spore counts were made after 10 days. The essential data are reported in table 3. Xylose, glucose, galactose, and sucrose were the best carbon sources for sporulation. Moderate sporulation was obtained on fructose and maltose. Sorbitol, mannitol, soluble starch, lactose, and sorbose were poor sources of carbon for sporulation. Neopeptone alone was a poor source of carbon for sporulation. Since glucose, fructose, mannose, galactose, and sorbose have the same empirical formula, the differences in

sporulation must be due to the configuration of the sugars. There was little, if any, correlation between radial growth and sporulation.

NITROGEN SOURCES

While other constituents of the glucose-neopeptone medium remained constant, the nitrogen sources were varied and used at a rate equivalent to 0.2866 gm. of nitrogen per liter. The results summarized in table 4 show

TABLE 4.—*The effect of different nitrogen sources with the glucose mineral-salt medium on sporulation of Colletotrichum lindemuthianum at 25° C. All nitrogen sources were used at a rate which supplied 0.2866 gm. of nitrogen per liter. (Harvest at 10 days)*

Nitrogen source	Diameter of colony (mm.)	Number of spores per colony (in millions)
Neopeptone[a]	47.5	557.00
Urea	33.0	213.00
Glycine	36.2	4.28
Asparagine	37.5	3.04
Arginine	21.5	1.71
Sodium nitrate	26.5	1.35
Ammonium sulfate	33.0	0.62
Casein hydrolysate	32.2	0.49

[a] Norit treatment reduced spore production to 26.14 millions per colony.

that neopeptone was the best source of nitrogen for sporulation; it was followed by urea, glycine, asparagine, and sodium nitrate. Hydrolyzed casein, ammonium sulfate, and aspartic acid were poor sources of nitrogen for spore production.

These media were also treated with Norit. As expected, the only significant reduction in amount of sporulation resulted when the glucose-neopeptone medium was so treated. The other nitrogen sources would be expected to contain only insignificant amounts of vitamins, if any. This is further evidence that the favorable effect of neopeptone on sporulation is due to the vitamins which it contains. Stokes, Gunness, and Foster (20) report neopeptone to contain 1.54 μg thiamin, and 0.09–0.27 μg biotin per gram.

CARBON–NITROGEN RATIO

A study was made of the effect of glucose concentration on sporulation when different sources of nitrogen were used in the medium. The amount of nitrogen used was 0.2866 gm. per liter in all media. It was found that the amount of glucose most favorable for sporulation varied with the nitrogen source used. With neopeptone, C/N ratios of 4:1, 8:1, and 16:1 were equally favorable for sporulation, but when C/N ratio was changed to 32:1, sporulation was reduced to one-sixth. A C/N ratio of 16:1 was best with sodium nitrate. With asparagine, highest yields of conidia were obtained with a 4:1 C/N ratio, but as the sugar concentrations increased there was a progressive decline in the amount of sporulation, until at C/N ratio 32:1 no spores were formed.

INITIAL pH OF SUBSTRATE

The pH values of the glucose-neopeptone medium were adjusted with lactic acid or sodium hydroxide. For pH values below 4.0 and above 7.0, the agar and the other constituents of the medium were autoclaved separately. Each Petri dish contained 30 ml. of the medium, and after inoculation the plates were incubated at 25° C. for 11 days, when spore counts were made in the usual manner. There is an increase (Fig. 1) in the number of conida from pH 3.1 to 3.4; then a sudden increase at pH 3.6, followed by a fall between pH 4.1 and 4.6; sporulation again increased and at pH 5.4 the maximum was reached. There was a gradual decrease in numbers of conidia produced as the medium became more alkaline. Several workers (7, 8, 9, 16) have reported the influence of pH on the sporulation of many fungi.

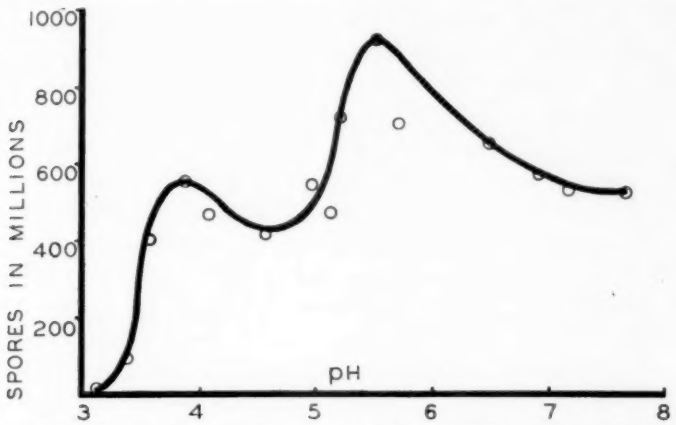

FIG. 1. The effect of initial pH on sporulation of *Colletotrichum lindemuthianum*.

MISCELLANEOUS OBSERVATIONS

Colletotrichum lindemuthianum produces variants of which only a few are stable. Several biologic forms (2, 11) and albino strains (3) have been reported. During the course of this investigation a number of saltants were isolated from a single-spore culture of the *gamma* strain. A few of these produced white or dark-colored mycelium, others formed acervuli with dark setae. All of these saltants sporulated abundantly, as did the *alpha* and *beta* strains and an isolate from West Virginia, when grown on the glucose-neopeptone medium at 20° C.

Variations of daylight, ultraviolet light, and aeration did not appear to influence sporulation.

DISCUSSION

Colletotrichum lindemuthianum produced more conidia on the best natural medium (glucose-neopeptone) than on the best semisynthetic medium (glucose-urea). However, not all natural media were superior to the best

semisynthetic medium. After the addition of agar (a natural substance of variable composition) to the media used for sporulation experiments, they were no longer strictly synthetic. It is believed, however, that the agar did not contain chemical factors which influenced sporulation of this fungus. In addition to the effects of specific constituents, the concentration of carbon and nitrogen sources was found to be important. Carbon/nitrogen ratios of 4:1 to 16:1 were found to be best when glucose and neopeptone were used; when the nitrogen source was changed, the optimum carbon/nitrogen ratio changed also. High concentration of glucose inhibited sporulation, whatever nitrogen source was used.

Neopeptone contains one or more substances which stimulate sporulation of this fungus. These substances were largely removed (Table 1) when the glucose-neopeptone medium was boiled briefly with Norit. The active materials adsorbed on the Norit were eluted with aqueous pyridine, and when added to the treated medium its original properties were largely restored. It appeared possible that these active substances were vitamins rather than amino acids since casein hydrolysate was a poor source of nitrogen for sporulation. Since the *gamma* strain of this fungus was known to be partially deficient for biotin and inositol, the effect of these and other vitamins on sporulation was tested. Inositol and biotin and thiamin singly and in combination replaced in part the substances removed by Norit from neopeptone. The effect of the vitamins on sporulation was most pronounced when the available nutrient supply was low. It may be doubted whether the vitamin content of neopeptone fully accounts for its excellence in stimulating sporulation.

Sporulation in many fungi takes place at the expense of reserve materials present in the vegetative mycelium, provided the latter is furnished with an adequate supply of vitamins which act as catalysts in the process leading to sporulation. This has been shown by Barnett and Lilly (1) for *Sordaria fimicola* which requires biotin for growth as well as for sexual reproduction, and by Magrou and Marneffe (14) for *Sphaerocybe concentrica* which requires thiamin for conidium production. Little information is available on the effect of vitamins on sporulation of partially deficient fungi. It should be noted that thiamin, which depresses the growth of the *gamma* strain of this fungus, markedly increased sporulation under certain conditions. We may suppose, therefore, that thiamin plays several roles in growth and reproduction, and that excess thiamin inhibits growth but not sporulation.

Stimulation in the production of conidia by an extract of spores and matrix is another interesting observation not recorded for any other fungus. Considering the similar behavior of spore matrix and vitamins which stimulate sporulation, it is possible that matrix contains certain vitamins including inositol and biotin, as well as other active substances. Stimulation of asexual reproduction in *Colletotrichum lindemuthianum* appears

to be another function of the spore matrix which was hitherto known only to preserve the viability of spores and help in their dissemination.

The glucose-neopeptone medium, which favored sporulation of the *gamma* strain, was also favorable for sporulation of three other isolates from nature, as well as some mutants which arose in culture.

SUMMARY

Colletotrichum lindemuthianum (*gamma* strain) does not sporulate abundantly on synthetic media. Not all natural media are equally satisfactory. A medium containing glucose, mineral salts, and neopeptone gave excellent sporulation. Sporulation on this medium was as good as that occurring on sterilized bean pods or bean agar. Glucose could be replaced by equivalent quantities of sucrose, xylose, or galactose without affecting the amount of sporulation. Mannose, maltose, fructose, and lactose were less favorable; while soluble starch, mannitol, and sorbitol were unfavorable. Neopeptone was by far the most favorable nitrogen source tested for sporulation. Other nitrogen sources, in decreasing order of effectiveness in stimulating sporulation, were urea, glycine, arginine, asparagine, and sodium nitrate.

There was no conidial formation below pH 3.0, but sporulation increased suddenly at pH 3.6, was greatest between pH 5.2 and 6.5, and moderate up to pH 7.7. Variations in light and aeration were without effect on sporulation.

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SOUR SKIN, A BACTERIAL ROT OF ONION BULBS

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In 1942 a bacterial scale rot of onion bulbs was described, common to New York State, and the pathogen was given the name *Pseudomonas alliicola*.[1] Since then another rot of onions known among the growers as "sour skin" and "slippery skin" has been brought to the attention of the writer by Dr. A. G. Newhall. The sour skin, which is the name most frequently used, is becoming prevalent in the onion-growing sections of the State and during the last two years many specimens have been received at harvest time and during early storage. At times some growers have claimed as high as 20 per cent loss, but this percentage probably includes other bulb rots.

The sour skin as observed on the Yellow Globe variety is a rot that attacks only certain of the outer fleshy scales of the bulb, although not necessarily the outermost scale. The inner scales that give rise to leaves have never been observed to be infected naturally or artificially. In double bulbs, however, the storage scales in the center of the onion between the two growing regions may become diseased. Infected scales do not have the watery and glassy appearance of the rot caused by *Ps. alliicola* but are slimy and yellow. The yellow color is caused partly by an aggregate of minute yellow masses under the epidermis of the scale and is probably a response of the plant; at least these masses are not composed of bacteria. Infected onions may exhibit a shrinkage of the upper portion of the bulb and in advanced stages of the disease the outer dry skin readily slips off during handling, while the portion of the bulb within the diseased slimy scales remains firm. Many growers think such onions have a sour, vinegar-like odor, from which the name of the disease is derived.

The appearance of the necrosis of the outer scales led one to believe that the disease is of bacterial origin, and microscopical examination of the tissue shows an abundance of bacteria. Fungi and yeast may also be present as secondary invaders. The latter organisms possibly may play some part in the sour odor attributed to the disease. Dilution plates made from necrotic tissue yield an abundance of bacterial colonies. These, however, are mainly saprophytes but probably this is to be expected from such disintegrated plant material. The two most common types of colonies to appear in the plates are first, smooth, white, rapidly growing, circular colonies, and second, pin-point colonies. Neither of these bacteria has proved to be pathogenic although numerous inoculations have been made with them on mature onion bulbs.

The true pathogen appears in rather limited numbers, at times only three or four to a plate and frequently scattered among the saprophytes.

[1] Burkholder, W. H. Three bacterial plant pathogens: Phytomonas caryophylli sp. n., Phytomonas alliicola sp. n., and Phytomonas manihotis (Arthaud-Berthet et Bondar) Viegas. Phytopath. 32: 141–149. 1942.

On beef-extract-peptone agar they are approximately 5 mm. in diameter, smooth and rough types, and a sulfur yellow in color. When transferred to tubes they grow readily on most media. When tops of onion bulbs are severed and smeared with these bacteria, infection takes place within a few days and progresses slowly but steadily down the outer fleshy scales. Reisolations from such artificially inoculated bulbs yield the same bacteria but again in limited numbers.

The sour skin has not been studied carefully in the field but inoculations of young growing onions in the greenhouse have produced no symptoms. As far as is known, only bulbs show the disease. Infection probably takes place when the onion tops are cut at harvest time, and it seems likely that the increased use of high-speed mechanical toppers may be contributing to the incidence of the disease. There is no evidence that the bacteria spread from infected bulbs to healthy ones in storage.

When inoculated into potato tubers, narcissus bulbs (King Alfred), and calla lilly rhizomes, the pathogen has not proved infectious. Light infection has been obtained on iris rhizomes but the spread of the rot in the plant is checked rapidly when a layer of cork tissue is formed.

THE PATHOGEN

The description of the pathogen which follows is based on seven isolates from different bulbs. Three were isolated in the Fall of 1947 and four in the Fall of 1948. All behaved similarly in culture although rough and smooth types are frequent in dilution plates.

Morphology. The pathogen is a non-spore-forming straight rod with rounded ends, occurring singly or in pairs. It varies in size from 1μ to 2.8μ in length with an average of 1.9μ. It is approximately 0.8μ in diameter. The bacterium is Gram-negative, frequently with two granules in a cell, and is motile with one to three polar flagella.

Cultural characters. On beef-extract-peptone-agar (Bacto) slants, growth is sulfur yellow, filiform, and butyrous to slightly viscid in consistency. Most cultures appear rough. A water-soluble, yellow to yellow-green pigment diffuses into the agar surrounding the growth. On potato-dextrose agar the colony color is pale yellow and there is no discoloration of the medium. Nutrient broth (Bacto) becomes turbid in 24 hr. and later a yellow pellicle is formed. Litmus milk may begin to clear in three days or later. Litmus is reduced and eventually the cleared medium becomes tan with a yellow pellicle. Clara's medium[2] becomes white and turbid but later there develops a slight bluish tinge. This is an indication that the water-soluble pigment produced on the nutrient agar is not the same as the green fluorescent pigment frequently observed in cultures of many *Pseudomonas* species. On Krumwiede's triple-sugar agar, growth is very abundant, yellow-green, and extremely wrinkled. The medium remains red.

Physiological studies. Growth in gelatin is moderate with a slow lique-

[2] Clara, F. M. A comparative study of the green-fluorescent bacterial plant pathogens. Cornell Agr. Exp. Sta. Mem. 159. 1934.

faction. Growth in tryptone broth is good but there is no production of indole or H_2S. Nitrates are reduced to nitrites. The pathogen utilizes for its nitrogen source ammonium salts, nitrates, and peptone. Asparagin is utilized as a combined carbon and nitrogen source, but tyrosine is not so used. The bacterium is urease negative,[3] and is slightly lipolytic.[4] In shake cultures of beef-extract-peptone agar plus one per cent dextrose all growth, pale yellow in color, is on the surface, showing that the bacterium is a strict aerobe.

The onion pathogen, when grown in a synthetic medium (Koser's medium minus the citrate), utilizes the following carbon sources: arabinose, xylose, dextrose, levulose, lactose, maltose, sucrose, glycerol, mannitol, and salicin; also the sodium salts of citric, hippuric, malonic, and tartaric acids. Growth is very slight in rhamnose, and 2 per cent ethyl alcohol is not used. Sodium ammonium pectate medium is not liquefied and starch is not hydrolyzed.

The pathogen is methyl red negative, and acetylmethylcarbinol is not formed.

Growth in beef-extract-peptone broth plus 3 per cent salt is good. There is no growth in the same medium plus 5 per cent salt.

The optimum temperature for growth is approximately 30° C.; maximum 42° C., and minimum below 9° C. At this latter temperature there is a light turbidity of the broth, but at 6° C. with no turbidity there is an accumulation of cells in the sediment over several weeks' duration.

In the previous article, published in 1942, the writer has given a review of the bacteria hitherto described as occurring on onions or causing diseases of this crop. The review will not be repeated here, but it may be stated that the pathogen under consideration in this article is not similar to any of those referred to previously. The pathogen and the disease it causes appear to be new to literature. The organism is therefore treated as a new species. The description of this bacterium as reported above places it in *Pseudomonas* as the genus stands at present. Certain characters, however, show that it does not fit in too well with the majority of the plant pathogens of this genus that are known as the green fluorescent group. The yellowish, water-soluble pigment is probably not the same as the green pigment, the sulfur-yellow color of the cells is unusual, the high temperature range of the "sour-skin" pathogen is much above that of the green fluorescent species, and the pathogen is more saccharolytic than this group. Other characters, however, especially that of polar flagellation, place the pathogen in this genus. The name **Pseudomonas cepacia** n. sp. is therefore proposed.

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[3] Stuart, C. A., E. von Stratum, and R. Rustigan. Further studies on urease production of Proteus and related organisms. Jour. Bact. 49: 437–444. 1945.

[4] Starr, M. P. Spirit-blue agar; a medium for detection of lipolytic microorganisms. Science 93: 333–334. 1941.

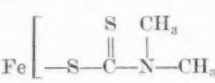
NOTICE

The Subcommittee on Fungicide Nomenclature of The American Phytopathological Society, cooperating with the Interdepartmental Committee on Pest Control, has selected common names for five commercially-available fungicidal chemicals which are useful in the control of various destructive plant diseases. Information concerning the names, the approval and acceptability of their use as coined names for these specific chemicals is filed with the Trade-Mark Division of the United States Patent Office, Department of Commerce, to preempt the use of the names as trade marks. Notices of the selection of the common names and definitions are being forwarded to officials and agencies in other countries.

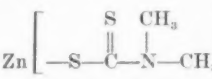
Selection of the common names was initiated by the Subcommittee and has been adopted by them on behalf of The American Phytopathological Society. This carries with it the acceptability of the names for use by that Society and its publications. The Interdepartmental Committee on Pest Control has consulted with various Governmental agencies and with collaborators. Contacts with various agencies and collaborators have indicated that they are considered acceptable for use. Those consulted include the Committee on Therapeutic Agents and Appliances of the American Veterinary Medical Association; American Medical Association; Association of Economic Poisons Control Officials; American Chemical Society through the Editor of CHEMICAL ABSTRACTS; and the following from the Department of Agriculture—Insecticide Division of the Production and Marketing Administration and the Bureau of Plant Industry, Soils, and Agricultural Engineering, which unit advises that the names will be acceptable in connection with publications that may be issued by that Bureau.

The coined common names and designations are:

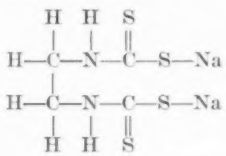
Ferbam for the fungicidal chemical, ferric dimethyl dithiocarbamate, which has the empirical formula, $FeC_9H_{18}N_3S_6$, and the following structural formula:



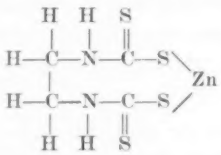
Ziram for the fungicidal chemical, zinc dimethyl dithiocarbamate, which has the empirical formula, $ZnC_6H_{12}N_2S_4$, and the following structural formula:



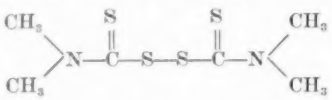
Nabam for the fungicidal chemical, disodium ethylene bisdithiocarbamate, which has the empirical formula, $Na_2C_4H_6N_2S_4$, and the following structural formula:



Zineb for the fungicidal chemical, zinc ethylene bisdithiocarbamate, which has the empirical formula, $ZnC_4H_6N_2S_4$, and the following structural formula:



Thiram for the fungicidal chemical, tetramethylthiuram disulfide, which has the empirical formula, $C_6H_{12}N_2S_4$, and the following structural formula:



The foregoing five words (ferbam, ziram, nabam, zineb, and thiram) are coined common names for specific chemicals. They should be used as common names. When written, the first letter should not be capitalized except where such capitalization is in keeping with the accepted use for common names.—S. A. ROHWER, Chairman, Interdepartmental Committee on Pest Control.